Herpesvirus and HIV-1 co-infection of human macrophages

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I, Rosemary Hughes confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

HSV-1 and HIV-1 co-infection of human macrophages represents a clinically relevant model with which to investigate the host-pathogen interactions between macrophages and viruses. In this thesis, I demonstrate that HSV-1 productively infects human monocyte derived macrophages, with associated cell death and type I IFN responses, and additionally, that a proportion of macrophages support latent HSV-1 infection. I define latency as the absence of lytic gene transcription, virion production and cell death, in the presence of persistent expression of the HSV-1 latency associated transcript (LAT). I also demonstrate that HSV-1 super-infection increases HIV-1 transcription, and that latent HSV-1 can be reactivated by HIV-1. HSV-1 latent infection of neurons is well established, but this is the first report, to my knowledge, of HSV-1 latent infection of myeloid lineage cells. The potential for macrophage reservoirs of latent HSV-1 may be an important factor for the clinical management of persistent reactive HSV-1 disease. Furthermore, HIV-1 infection *in vivo* is known to increase the frequency of HSV-1 reactivation in the host through the indirect mechanism of immune system suppression. However, a direct interaction between cells latently infected with HSV and HIV-1 has not previously been observed. Reactivation of latent HSV by HIV-1 therefore provides a novel mechanism for the well-established clinical synergy between these viruses.

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Abbreviations

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6-HD	6-hydroxydopamine.

A

ACV	acyclovir.
ADAR1	adenosine deaminase acting on RNA.
ADV5	adenovirus 5.
AIM2	absent in melanoma 2.
AP-1	activator protein-1.
ASC	apoptosis-associated Speck-like protein containing a caspase activation and recruitment domain.
ATP	adenosine triphosphate.

B

BM	bone marrow.
BVdUrd	(E)-5-(2-bromovinyl)-2'-deoxyuridine.

C

cAMP	cyclic adenosine monophosphate.
CCL2	chemokine (C-C motif) ligand 2.
CCL3	chemokine (C-C motif) ligand 3.
CDK1	cyclin-dependent kinase 1.
cGAMP	cyclic guanosine monophosphateadenosine monophosphate.
cGAS	cyclic GMP-AMP synthase.
cIAP	cellular inhibitor of apoptosis.
CNS	central nervous system.
CoREST	REST corepressor 1.
CpG DNA	cytosine and guanine rich DNA.
CTD	carboxyterminal domain.
CXCL10	C-X-C motif chemokine 10.
cyt-c	cytochrome c.

D

DAI	DNA-dependent activator Of interferon.
DAI	DNA-dependent activator of IFN-regulatory factors.
DC	dendritic cell.
DCPE	2[[3-(2,3-dichloro-amino]-ethanol.
DDB1	damage-specific DNA binding protein 1.
DNA	deoxyribonucleic acid.
dNTP	deoxynucleoside-triphosphate.
DR	death receptor.
DRG	dorsal root ganglion.
dsDNA	double stranded DNA.
dsRNA	double-stranded RNA.

E

E	early.
EBV	Epstein-Barr virus.
eIF	eukaryotic initiation factor.

F

FADD	fas-Associated protein with death domain.
Fig.	figure.
FISH	fluorescence *in situ* hybridization.

G

gB	glycoprotein B.
gC	glycoprotein C.
gD	glycoprotein D.
gL	glycoprotein L.
gM	glycoprotein M.
GM-CSF	granulocyte macrophage colony-stimulating factor.

H

H2B	histone 2B.
HAART	highly active antiretroviral therapy.
HCF-1	host cell factor 1.
HCMV	human cytomegalovirus.
HDAC-1	histone deacetylase 1.
HFF	human foreskin fibroblast.
HIV	human immunodeficiency virus.
HMGB	high-mobility group protein B.
HPV	human papillomavirus.
HSV	herpes simplex virus.

I

ICP	infected-cell polypeptide.
IE	immediate early.
IFI16	γ interferon inducible protein.
IFN	interferon.
ifna	interferon α gene.
ifnb	interferon β gene.
IFNR	interferon receptor.

IKK	inhibitor of NF-κB kinase.
IL-1β	interleukin.
IP10	interferonγ-induced protein 10.
IPS-1	interferon-β promoter stimulator 1.
IRAK	interleukin-1 receptor-associated kinase.
IRF	interferon regulatory factor.
ISG	interferon stimulated gene.

K

K$^+$	potassium $+1$ ion.
kb	kilobases.
KSHV	Kaposi's sarcoma-associated herpesvirus.

L

L	late.
LAT	latency assoicated transcript.
LC	Langerhan cell.
LPS	lipopolysaccharide.
LRRFIP1	leucine rich repeat in FLII interacting protein 1.
LTNP	long term non-progressor.

M

M-CSF	macrophage colony-stimulating factor.
MCMV	mouse cytomegalovirus.
Mda5	melanoma differentiation-associated protein 5.
MEF	mouse embryonic fibroblasts.
MLV	murine leukemia virus.
MoDC	monocyte derived dendritic cell.
MPS	mononuclear phagocyte system factor.
Mx	myxovirus resistance protein.
MyD88	myeloid differentiation primary response gene 88.

N

NAD	nicotinamide adenine dinucleotide.
NF-κB	nuclear factor κ-light-chain-enhancer of activated B cells.
NGF	nerve growth factor.
NLRC	NLR family CARD domain-containing protein 4.
NLRP	NACHT, LRR and PYD domains-containing protein.

P

PACT	PKR activator.
PAMP	pattern associated molecular pattern.
PARG	poly ADP ribose glycohydrolase.
PARP	poly ADP ribose polymerase.
PBMC	peripheral blood mononuclear cells.
pDC	plasmocytoid dendritic cell.
PI3K	phosphoinositide 3-kinase.
PK	protein kinase.
PMA	phorbol 12-myristate 13- acetate.
pol III	DNA dependent RNA polymerase III.
poly(dA:dT)	poly(deoxyadenylic-deoxythymidylic) acid.
poly(dC:dG)	poly(deoxyguanylic-deoxycytidylic).
poly(I:C)	polyinosinic:polycytidylic acid.
pppRNA	5' triphosphate dsRNA.
PRR	pattern recognition receptor.

R

Ref.	references.
REST	RE1-silencing transcription factor.
RIG-I	retinoic acid-inducible gene 1.
RLR	RIG-I like receptor.
RNA	ribonucleic acid.
RNAPII	RNA polymerase II.
RT	reverse transcriptase.

S

SAMHD1	SAM domain and HD domain-containing protein 1.
SCG	superior cervical ganglion.
SIV	simian immunodeficiency virus.
ssDNA	single-stranded DNA.
ssRNA	single-stranded RNA.
STAT	signal transducer and activator of transcription.
STING	stimulator of interferon genes.

T

T3SS	type III secretion system.
TAR	transactivation response RNA element.
TBK1	tank-binding kinase-1.
TCR	T-cell receptor.
Tfh	T follicular helper.

TK	thymidine kinase.
TLR-	toll like receptor.
TNF-α	tumor necrosis factor-α.
TNFR	tumor necrosis factor-α receptor.
TRAF	TNF receptor associated factor.
TRAIL	TNF-related apoptosis-inducing ligand.
TRBP	TAR RNA binding protein.
TRIF	TIR-domain-containing adapter-inducing interferon-β.
TRIM	tripartate motif protein.

U

UV	ultraviolet.

V

vhs	virion host shutoff protein.
VSV	vesicular stomatitis virus.

W

WHO	World Health Organization.

Z

ZBP-1	Z-DNA binding protein-1.

1 Introduction

1.1 Aims

In this thesis I aim to characterize herpes simplex virus-1 (HSV-1) and human immunodeficiency virus-1 (HIV-1) co-infection of human macrophages. Cellular responses to viral infection are highly cell-type, species and pathogen specific(1–4). Consequently, to fully understand the host response to virus infection, research using an infection model of a clinically relevant pathogen and cell-type is essential. Macrophages are important cells of the innate immune system and can be infected by both HIV-1 and HSV-1(5–9). From a clinical perspective, macrophages are important cellular reservoirs of HIV-1 infection and are present at HSV-1 lesions(9–13). Furthermore, HSV-1 and HIV-1 interact at both the clinical and epidemiological level(14,15). Therefore, HSV-1 and HIV-1 co-infection of human macrophages represents a clinically relevant model with which to investigate the host-pathogen interactions between macrophages and viruses, and the potential interactions of HIV-1 and HSV-1 at the cellular level.

1.2 Summary

In this introduction I outline the scientific literature regarding macrophages, innate immune responses, HSV-1 and HIV-1. I describe the cellular immunology of macrophages; the principles of the innate immune response to viruses, including pathogen detection, type I interferon (IFN) responses and cell death; basic HSV-1 and HIV-1 virology and the interactions of HSV-1 and HIV-1 with the innate immune system, macrophages and each other.

1.3 Macrophages

The innate immune system is the first line of defence against infection(16). Components of innate immunity detect infection, mount rapid inflammatory and antiviral responses and orchestrate adaptive immunity(17,18). Macrophages are important cells of the innate immune system. They are tissue resident sentinels(5,6) responsible for eliciting cellular defence mechanisms and recruiting and activating components of adaptive immunity(7). They are also target cells for a variety of intracellular pathogens including HIV-1(8,9), *Mycobacteria tuberculosis*(19), *Leishmania*(20) and human cytomegalovirus (HCMV)(21).

Macrophage phenotypes are diverse and tissue specific(5), ranging from the Kupffer cells of the liver(22) to the microglial of the brain(23). Macrophages also alter their phenotype in response to inflammatory stimuli(24–26). Transcriptome analysis has highlighted the diversity of transcriptional states macrophages occupy, a diversity that mirrors the signals that they receive(25,27,28).

Macrophages, along with dendritic cells (DCs) and Langerhan cells (LC), are part of the mononuclear phagocyte system (MPS). Traditionally, macrophages were thought to be derived from a temporal series of progenitor cells produced in turn by the yolk sac, fetal liver and bone marrow (BM)(29). The BM produces circulating monocytes that differentiate into macrophages within the tissue(30). However, there is now evidence suggesting that, at least in mice, the majority of tissue macrophages are derived from the yolk sac(31). The BM produces circulating monocytic progenitors that differentiate into either DCs or macrophages in the tissue, and it is only these macrophages that are replaced, whereas yolk sac derived macrophages persist throughout life(32). However, it is not known which, if either, of these models correspond to human macrophage ontology.

Primary macrophages can be extracted from tissue(19). However, these macrophages are subjected to significant stress that may alter their phenotype so it is unclear how closely they resemble macrophages *in vivo*(5,19). In the tissue, infiltrating monocytes are exposed to a spectrum of growth factors that induce differentiation into macrophages(33). Monocytes from the peripheral blood can be differentiated *in vitro* into tissue macrophage-like cells using growth factors such as macrophage colony-stimulating factor (M-CSF) and/or granulocyte macrophage colony-stimulating factor (GM-CSF)(9). The phenotype of the resulting macrophages is dependent on the growth factor(s) used. Indeed, M-CSF and GM-CSF bind different receptors, use different downstream signalling pathways(26,33) and elicit differential responses in cells of the MPS(34–36). Based upon cytokine expression, GM-CSF differentiated macrophages have a pro-inflammatory phenotype whereas M-CSF differentiated macrophages have an anti-inflammatory or ‘resting’ phenotype(26,28). M-CSF is produced in steady state whereas GM-CSF is an inflammatory cytokine associated with IFN-γ production(37,38). Additionally, whereas M-CSF deficiency is associated with a lack of macrophages, GM-CSF deficiency is associated with alveolar proteinosis but not necessarily fewer macrophages(39). Taken together these data suggest that M-CSF is responsible for macrophage differentiation and that GM-CSF modulates macrophage function(33,40).

1.4 Pattern recognition receptors

The innate immune system uses germ-line encoded receptors to detect invading pathogens(17). A broad range of pathogen associated molecular patterns (PAMPs) are detected by these pattern recognition receptors (PRRs), including components of bacterial and fungal cell walls, pathogen nucleic acids and viral glycoproteins(41,42). Engagement of a PRR with its PAMP can lead to a broad range of intracellular signalling events and cellular responses, including inflammatory cytokine release, induction of a type I IFN response, establishment of an antiviral state, induction of programmed cell death and post-translation responses such as inflammasome activation(43).

Macrophages express a broad array of PRRs able to detect viral infection(6). Many of these detect intracellular foreign and inappropriately localised nucleic acids indicative of viral infection. These PRRs include the endosomal toll-like receptors (TLRs) and cytostolic receptors from the retinoic acid-inducible gene 1 like receptor (RLR) and PYHIN protein families. In particular, TLR3, 7 and 9 detect double-stranded (ds)RNA, single-stranded (ss)RNA and cytosine and guanine rich DNA (CpG DNA) respectively(44). The cytosolic RLR family members RNA sensors retinoic acid-inducible gene 1 (RIG-I) and melanoma differentiation-associated protein 5 (Mda5), detect short 5’triphosphate (5’PPP) RNAs and ssRNA respectively(45,46) (Fig.1). In addition, there are many cytosolic DNA sensors, including Z-DNA binding protein-1 (ZBP-1)(47), absent in melanoma-2 (AIM2)(48), γ-interferon-inducible protein 16 (IFI16)(49) and the recently discovered cyclic GMP-AMP synthase (cGAS)(50). The PRRs that detect nucleic acid are summarized in Table 1 and Figure 1.

Currently, it is unclear why there are so many sensors of cytoplasmic nucleic acid. It is likely that nucleic acid sensors operate in both a cell and pathogen dependent manner. Consequently, investigating the function of these sensors in a immunological relevant cell type, such as macrophages, may provide novel insights into the specificity of their function. This is particularly the case for cells that are targets for clinically important viruses such as HSV-1 and HIV-1.

1.5 PRR signalling

Ligation of a PRR can induce expression of type I IFN. IFNβ and IFNα transcription are dependent on interferon regulatory factor (IRF)3 and/or IRF7 phosphorylation, dimerization, nuclear translocation and binding to the IFNβ gene (*ifnb*) promoter (Fig.1). The signalling pathways leading to IRF3/IRF7 activation vary considerably(51,52), for example, TLR3 associates with TIR-domain-containing adapter-inducing IFNβ (TRIF)(44), RIG-1 and Mda5 bind IFNβ promoter stimulator-1 (IPS-1)(53), leading to the assembly of a signalling complex composed of TNF receptor associated factor (TRAF)-3, tank-binding kinase-1 (TBK1) and inhibitor of NF-κB kinase (IKK)-ϵ(54,55), whilst cGAS produces atypical cyclic guanosine (cGAMP), a secondary messenger that activates stimulator of IFN genes (STING) at the mitrochondrial membrane(56). There is also considerable cross-talk between the signalling pathways involved in induction of type I IFN expression, up-regulation of inflammatory cytokines and activation of cell death signalling. NF-κB for example can be involved in all three of these processes(57), and is necessary for *ifnb* transcription in some but not all contexts(58,59).

1.6 Type I IFN

Interferons are divided into type I and type II, and subdivided within these classes based on sequence homology, molecular structure and receptor usage(60,61). The IFN classes are summarised in Table 2. Type I IFNs are encoded by multiple *ifna* genes, one *ifnb* gene and the more recently identified ϵ, κ type I IFN encoding genes(60,62).

Type I IFNs can be produced by almost any nucleated cell. Following its release, IFN-β binds the heterodimeric interferon receptor complex (IFNAR1/IFNAR2) in an autocrine or paracrine manner. This initiates a series of intracellular phosphorylation events, involving the adaptor proteins signal transducer and activator of transcription (Stat)2, tyrosine kinase 2 (Tyk2) and Janus kinase 1 (Jak1) (Fig.1). Phosphorylation of these adaptors provides a docking site for Stat proteins that are then activated via the kinase activity of the IFNAR1 complex(62,63). Phosphorylated Stats form hetero- and homodimeric transcriptional activator complexes that translocate to the nucleus, drive expression of IFNα and interferon stimulated genes (ISGs) and modulate the expression of hundreds of other genes(62). The type I IFN response is turned off by the activity of suppressor of cytokine signalling (SOCS3) protein family members, for example SOCS3 associates with the IFNR, inhibits Jak activity and promotes Jak proteosomal degradation(64–66).

ISG expression establishes a cellular antiviral state that both enables a cell to restrict intracellular virus replication and protects bystander cells from viral infection(67). As such, the type I IFN response is the main weapon of the innate immune system against viral infection, as highlighted by the extensive array of strategies viruses use to evade detection by PRRs, induction of type I IFN and type I IFN inducible signalling events(68). A ‘non-classical’ pathway for activation of ISGs by type I IFN also exists, involving the intracellular signalling mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) pathways and regulation at the level of ISG messenger RNA (mRNA) translation(60,62,69).

1.7 ISGs

The activities of a number of ISGs have been well characterised. Many, such as protein kinase R (PKR), have generalised antiviral effects(70,71). These include the cytosolic enzymes activated by dsRNA, such as PKR and 2-5-oligoadenylate (2-5(A))-synthetases (OAS)(70–73), the myxovirus resistance protein (Mx) and Guanylate binding proteins that interfere with virus replication by inhibiting nuclear transport of viral nucleocapsids(74), and ISG56 family members that inhibit host cell protein synthesis via inhibition of eukaryotic initiation factor (eIF)-3 function(75).

dsRNA induces dimerization and autophosphorylation of PKR(76), leading to PKR mediated phosphorylation of the α subunit of the eIF-2 complex(77). This results in general translation shut-off, induction of cell death(78) and inhibition of virus replication(76). PKR activation is highly regulated, for example by cellular TAR RNA binding protein (TRBP), adenosine deaminase acting on RNA (ADAR1) and PKR activator (PACT). During HIV-1 infection PACT inhibits PKR activation(79), whereas HSV-1 Us11 protein inhibits PACT mediated PKR activation(80).

OASs are nucleotidyltransferases that produce 2'-5'-oligoadenylates. These bind and activate RNase L, which then mediates degradation of viral and cellular RNA(81,82). Interestingly cGAS, the recently discovered DNA sensor, is an OAS homologue. The functional similarity of cGAS and the OAS system has recently been reviewed(83). Full activation of OASs in virally infected cells leads to the inhibition of protein synthesis and induces apoptosis(84). HSV-1 Us11 also inhibits OAS activation, late during viral replication(85).

Mx proteins are dynamin-like large GTPases, best known for inhibiting the replication of negative-stranded RNA viruses, such as influenza(74). However, they also have activity against other virus types. The antiviral role of Mx proteins appears linked to their ability to detect nucleocapsid structures in the cytoplasm(86).

Other ISGs have effects limited to certain virus types, for example retrovirus restriction factors such as apolipoprotein B mRNA-editing enzyme-catalytic polypeptide-like (APOBEC)3G/3F, tripartate motif protein (TRIM5)-α and tetherin(87–90), although these can also have activity against other viruses including HSV-1(91–94).

The APOBEC family of cytidine deaminases catalyze the deamination of cytidine to uridine in single-stranded nucleic acids. They interfere with retroviral replication via hypermutation of the viral genome, inhibition of reverse transcription, and inhibition of provirus integration into the host genome. APOBEC3DE, APOBEC3F, APOBEC3G, and APOBEC3H all have anti-retroviral activity(95). HIV-1 Vif counteracts the activity of the APOBEC restriction factors by a variety of mechanisms, including inducing the formation of a E3 ubiquitin ligase complex to direct degradation of APOBEC proteins(96–101), depleting the intracellular pool of ABOBEC3G by directly binding ABOBEC3G mRNA and down-regulating its translation and stability(98,102,103) and directly inhibiting ABOBEC3G deamination activity by altering its processive DNA scanning mechanism(104). APOBEC proteins do have activity against herpesviruses, including HSV-1, but it is not yet known whether HSV-1 encodes APOBEC antagonists(94).

Tetherin prevents the release of virus particles after they have budded from infected cells. HIV-1 is able to counteract the activity of tetherin(105–107). Recently, tetherin was also shown to have activity against HSV-1(91,92) and HSV-1 gM is able to counteract this effect.

Many more ISGs have yet undefined functions or functions that do not appear related to antiviral immunity (108). Modulation of these genes may reflect the immuno-regulatory role of type I IFN (109).

Type I IFNs can have global effects on the innate immune system (110), for example, they can promote NK cell-mediated and CD8+ T cell mediated cytotoxicity (111), trigger apoptosis of virally infected cells (112) and enhance maturation, cross-presentation, antigen presentation and migration of dendritic cells (DCs), thereby promoting activation of the adaptive immune responses (113,114). Type I IFN can also enhance Th1 cell responses and promote the generation of T follicular helper (Tfh) cells (115–117).

1.8 Cell death

Ligation of PRRs can lead to activation of programmed cell death pathways (118). Death of an infected cell can have advantageous consequences for the host, such as eliminating cellular reservoirs of viral infection (119–121), preventing high-jacking of cellular machinery for the purposes of viral gene expression, genome replication and protein expression (122,123), and alerting the immune system to danger (124,125). Many viruses encode or activate cell survival factors in order to counteract the cell death response early during viral infection (126–129). However, cell death can also be advantageous for a virus, for example, by facilitating virion release and eliminating cells alerted to infection (130). Consequently, the interaction between cell death responses and viral replication can be complex (131).

Cell death can occur by apoptosis, pyroptosis, necroptosis or necrosis (132–135). These cell death pathways can be distinguished on the basis of the activation signal, the nuclear morphology and plasma membrane integrity of the dying cells, the activity of specific cell death inhibitors and the cell-types in which they occur (136,137). Necrosis is an unprogrammed form of cell death characterised by cellular swelling, membrane blebbing, complete breakdown of the plasma membrane and release of cytoplasmic contents into the extracellular fluid (137). Pyroptosis is defined as caspase-1 dependent cell lysis (138) and was first described in *Salmonella typhimurim* and *Shigella* infected macrophages (139,140). Pyroptosis is activated via the formation of inflammasome complexes and is often accompanied by interleukin (IL)-1β and IL-18 release (141) (Fig.2). Apoptosis is defined as the caspase-dependent breakdown of the cell components into apoptotic bodies (142). Apoptotic cells undergo morphological changes characteristic of only this form of cell death, these include membrane blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation and chromosomal DNA fragmentation. Apoptosis can be activated by the extrinsic pathway, via death receptors on the cell surface, or the intrinsic pathway (143) via mitochondrial dysfunction and cytochrome c (cyt c) release (144). Death receptors include the TNF-α receptor and Fas receptor. Finally, necroptosis is a cell death pathway involving RIP3 and/or RIP1 signalling (128,145), in the absence of caspase-8 activation (Fig.3) (146–148). It has a role in development and immune responses (128,145,148,149), and is often activated following inhibition of apoptosis, pyroptosis or both (146–148). The morphological characteristics, activating signals and receptors and cell-types in which each cell-death pathway can occur are summarized in Table 3. There can also be considerable interaction between the cell death pathways, for example LPS activation of macrophages redirects *Yersina* induced macrophage cell death to caspase-1 dependent pyroptosis from caspase 8/3, cyt c dependent apoptosis (150).

1.9 Herpesviruses

Herpesviruses are among the largest and most complex of human viruses(151). Herpes virions are relatively large, 100-200nm in diameter, enveloped and contain a double-stranded DNA viral genome within a icosahedral capsid formed of viral protein(152,153). Herpesvirus genomes include 70-200 predicted open reading frames (ORFs)(154), providing the genetic diversity and complexity that enables herpesviruses to both manipulate the host cell environment and the immune system(155). Herpesviruses have a highly co-ordinated pattern of gene expression that involves both temporally regulated recruitment of cellular transcription complexes and epigenetic manipulation(156–158).

A characteristic of all herpesviruses is their capacity to establish a long-term latent infection in their host(159). In some host cells a static state of viral latency is established involving the repression of lytic gene expression, the expression of latency associated viral factors and persistence but not replication of the viral genome(160–162). In other cells, lytic viral gene expression occurs, leading to genome replication, the production of nascent virions, virion release and cell death. As described in detail below with respect to HSV-1, the tropism of herpesviruses, as well as their capacity to establish a latent infection, is determined by the cell receptors expressed and intracellular conditions of the host cell(151). These intracellular conditions can include the differentiation state of the cell, whether the cell is activated against viral infection by, for example, type I IFN stimulation, and whether the cell is in a pro or anti-apoptotic state(151).

1.10 HSV-1

HSV-1 belongs to the α-*herpesviridae* sub family, of which varicella zoster virus (VZV) and HSV-2 are also members. Viruses in this family typically have narrow species tropism and broad cell tropism. They generally target neurons for long term residence, but also replicate in epithelial cells - a stage necessary for efficient virion transmission from the skin or mucosa. HSV-1 is typically associated with cold sores, or blisters, on the lips. HSV-2 is associated with genital blisters. However, both viruses can cause lesions at either site, and HSV-1 is becoming the leading cause of genital herpes in the developed world(163).

Following transmission to the mucosal epithelium, an HSV-1 lesion appears a few days after infection. At the lesion, infected epithelial cells release virions and die. HSV-1 virions then enter sensory neurons innervating the site of transmission, are transported up the nerve axon to the nucleus and establish a life-long latent infection in the neuron. Latent HSV-1 re-activates throughout life, as a result of external stimuli such as stress, cold and immune-system repression. Re-activation can result in assymptomatic virus shedding or HSV-1 lesions. The HSV-1 replication cycle is represented schematically in Figure 4.

HSV-1 has a global prevalence of 70-90%, as determined by seropositivity(163). 50% of individuals who experience symptomatic HSV-1 primary infection will have recurrent HSV-1 disease(164), although the symptoms of primary infection are generally more severe than recurrent infection. Cold sores are the most common type of HSV-1 lesions. However, HSV-1 can also cause diseases associated with the skin, eyes and brain.

HSV-1 cutaneous lesions occur around the skin of the nails and this used to be a common problem for dentists working without gloves(165). HSV-1 infection of the eye can cause keratitis and blindness(166), and HSV-1 lytic replication in the brain can lead to encephalitis and meningitis(167). Herpes simplex encephalitis (HSE) causes significant mortality and neurological morbidity(168). HSV primary genital infection is associated with foetal loss, premature labour and neonatal HSV infection(169). Neonatal HSV infection is rare but has high morbidity and mortality. Symptoms range from isolated skin, eye and mucous membrane disease, to central nervous system (CNS) disease(170–172). Severe HSV-1 primary disease and recurrent infections are also a significant risk for immuno-compromised patients. Complications of HSV-1 infection in acquired immunodeficiency syndrome (AIDS) and transplant patients can include pneumonitis and hepatitis(4,173–175).

The majority of HSV-1 disease can be treated with acyclovir (ACV). ACV is a guanosine analog that acts as an antimetabolite, inhibiting HSV-1 genome replication. Prophylactic ACV treatment can also be used to reduce the risk of recurrent HSV-1 lesions and infection in solid organ and haemotatic stem cell transplant patients. However, the overall management of HSV disease is complex, due to the variability of the host-pathogen interactions involved(171), for example it is often unclear whether the immune response to HSV-1 infection is beneficial or harmful to the host, and the ability of HSV-1 to establish a latent infection means that it is currently impossible to eradicate it from the host. An over-active immune response to HSV-1 infection underlies the pathogenesis of HSV-1 encephalitis, whereas HSV-1 reactivation from latency is often associated with immune-suppression. It is generally accepted that there is a need for a greater understanding of HSV-1 pathogenesis to aid future research into targeted drug therapies and vaccine development(176).

The molecular virology of the HSV-1 lytic and latent replication cycles have been well characterized in tissue cultured epithelial cells, animal models and cultured neurons. In epithelial cells HSV-1 lytic replication occurs by tightly regulated sequential expression of viral immediate early (IE, α), early (E, β) and Late (L, γ) genes. Following virion entry into an epithelial cell, the virion protein VP16 is transported to the nucleus along with the viral genome. VP16 activates expression of the IE gene infected cell polypeptide (ICP)0 by unravelling the HSV-1 episomal genome from the heterochromatin state that host cell factors would otherwise establish. ICP0 activates expression of the other IE proteins within 2-4 hours of infection, by sequestering the transcriptional repression complex REST corepressor 1 (co-REST)/RE1-silencing transcription factor (REST)/host cell factor 1 (HCF-1). IE proteins induce expression of the E genes within 4-8 hours post infection. These genes include viral DNA polymerase (DNA Pol) and virus thymidine kinase (TK), and have roles in viral DNA replication and promoting cell survival, activating L gene expression and suppressing IE gene expression. L viral genes are divided into two types, leaky late (γ1) and true late (γ2) genes. Leaky late genes include glycoprotein B (gB), ICP34.5, gD and ICP5 and are expressed independently of viral genome replication(177). True late genes are expressed 8-12 hours post infection and form the structural components of nascent virions, activate virus release and promote cell death(178). The processes of HSV-1 gene expression are represented schematically in Figure 5.

1.11 HSV-1 latency

The major site of HSV-1 latency is the ganglionic sensory neurons, primarily the trigeminal ganglia (TG) and the sacral dorsal root ganglia (179–181). However, latent HSV-1 has also been detected in the human adult nodose ganglia and vagus nerve (182,183).

There are three steps involved in the establishment of HSV-1 latency (184). First, acute infection at the mucosal epithelium leads to the production of a high amount of infectious virus. This is followed by entry of virions into sensory neurons. Viral gene expression is then extinguished, with the exception of the HSV-1 latency associated transcript (LAT), as productively infected cells die. HSV-1 latency is thought to occur when activation of ICP0 expression by VP16 and ICP0 dependent activation of IE gene expression is stalled (185). During productive infection VP16 induces IE gene expression by recruiting cellular transcription factors, including HCF-1, Oct1, lysine specific demethylase-1 (LSD1), and CLOCK histone acetyl transferase, such that the IE promoter is demethylated and transcription activated (185,186). In neurons, VP16 and the cellular HCF-1, are not translocated to the nucleus. IE transcription is therefore not activated and the viral genome is suppressed. The blocks to HSV-1 replication that can lead to latent infection are represented schematically in Figure 6.

During latency, the HSV-1 LAT accumulates, promoting cell survival and dampening down IE and E expression. Latent HSV-1 can be re-activated from the neurons by external stimuli, such as stress or immuno-suppression. During reactivation, infectious HSV-1 virions can be isolated from the TG and/or eye and nose swabs. It is not known whether latently infected neurons in which productive HSV-1 infection re-activates, survive and re-establish latency, or are killed.

Experimentally, there are two recognised forms of HSV-1 latency: "canonical latency", defined as the presence of episomal, circularized viral DNA in the absence of viral protein expression, DNA replication and virion production; and 'non-canonical latency' or quiescent infection, where HSV-1 replication is blocked at some point after IE gene expression, but prior to late gene expression and virion production.

In any assessment of HSV-1 infection, it is important to determine how HSV-1 replication proceeds, and to distinguish between lytic replication, canonical latency and quiescent infection. This requires assessments of HSV-1 infection at the level of IE, E and L gene and protein expression, virion production and cell death.

The molecular mechanisms of HSV-1 latency *in vivo* remain poorly understood. This is partly due to the difficulty of assessing what is essentially the lack of replicative infection *in vivo* and the inadequacy of *in vitro* models. It is impossible to subject cells in tissue culture to the same stresses that a body can receive. Additionally, an infection aborted due to the lack of ICP0 expression by a ICP0$^-$ virus, for example, may not be a realistic representation of latent HSV-1 infection of neurons. Consequently, there is considerable opportunity for improving our knowledge of HSV-1 latent infection.

1.12 HSV-1 in vitro

Multiple HSV-1 strains have been isolated and studied, including strain 17, KOS, strain F and SC16. HSV-1 is less susceptible to genetic mutation than other herpesviruses (187). However, there are still significant genetic differences between many HSV-1 laboratory and wild-type strains. These are often in genes encoding proteins that are essential for *in vivo* pathogenesis, but not for growth in tissue cell lines, for example the HSV-1 thymine kinase gene(188). Additionally, during HSV-1 genome replication, homologous recombination can occur(154,189). Consequently, laboratory strains of HSV-1 that have undergone multiple passaging, may give misleading data regarding the host pathogen interactions between HSV-1 and non-tissue culture cells, such as monocyte derived macrophages. Highly passaged HSV-1, for example, may have lost the capacity to interfere with type I IFN responses.

1.13 PRR detection of HSV-1

Multiple HSV-1 PAMPs and HSV-1 specific cellular PRRs have been identified. HSV-1 PAMPs include virion surface glycoproteins, the C-G rich viral genome, tegument protein and viral RNA(190). The signaling pathway activated in response to HSV-1 infection will be highly dependent on the PRR(s) activated. Detection of HSV-1 can lead to type I IFN signaling, release of inflammatory cytokines, and NF-κB, p38 and AP-1 signaling. PRRs that detect HSV-1 PAMPs are summarized in Table 4. As with PRRs in general, it seems odd that so many cellular proteins are needed to detect HSV-1 infection. It is possible that there is some redundancy in the system. Alternatively, it may be that these receptors function in a cell specific manner. One group has shown, for example, that HSV-1 can be detected by Mda5 but not by Pol III in macrophages(191). Consequently, yet again there is considerable opportunity to investigate HSV-1 detection in a clinically and immunologically relevant cell model.

1.14 HSV-1 and type I IFN

Detection of HSV-1 PAMPs by cellular PRRs leads to induction of a type I IFN response(192–195). Type I IFN has well-established anti-viral effects against HSV-1, but HSV-1 is also able to counteract these effects in a context dependent manner. IFN-β is highly expressed in the TG of HSV-1 infected mice(192,196) and has been proposed to play an important role in HSV-1 disease(196).

Type I IFN has an antiviral effect against HSV- 1 in both tissue culture(197,198) and the TG(196). In mouse models of HSV-1 infection, type I IFN increases cell survival and decreases virion production(196). In both HeLa cells and mouse macrophages, type I IFN can block HSV-1 infection prior to IE gene expression(199–201). In a mouse model lacking the IFNR, footpad or ocular inoculation where normally the infection would be controlled leads to systemic dissemination of the virus(202). Administration of interferon promotes survival of HSV-1 infected mice(195,203–205). Finally, a partial reduced ability to produce type I IFN in response to TLR3 stimulation, resulting from TLR3 deficiency, can lead to herpes simplex encephalitis (HSE) whilst the patient remains resistant to other infections(206,207). However, type I IFN also plays a role in immuno-pathogenesis during HSV-1 infection and type I IFN blocking antibodies or the genetic lack of the IFNR reduces the severity of HSV-1 disease in mice(208).

HSV gene products can counteract the host interferon response, for example by decreasing transcription of the ISG PKR and reducing Jak-1 and Stat-1 phosphorylation(196,209), or preventing the nuclear accumulation of IRF3 prior to *infb* transcription(210,211). HSV-1 also increases transcription of the negative regulator of type I IFN responses, SOCS3, in cell cultures treated with IFN-β(196,212,213).

1.15 HSV-1 and cell death

HSV-1 can both cause and inhibit cell death. *In vitro* HSV-1 is a highly lytic virus and can be used *in vivo* as a cytolytic tool during cancer therapy(214–219). Conversely, like all DNA viruses, HSV-1 possesses multiple strategies to maintain host cell survival throughout viral replication(220) and inhibition of cell death by HSV-1 is believed to be important for the establishment and maintenance of viral latency(221,222).

The separation of HSV-1 research into the investigation of either pro-death or anti-death signalling, by cancer researchers and molecular virologists respectively, has lead to confusion and conflicting results within the literature. However, the fundamental question of whether HSV-1 is pro-death or anti-death may be misleading. There is growing appreciation that a balance between pro-survival and pro-death signalling is established within an HSV-1 infected cell, and that this is dependent upon the cell type and the stage of the viral replication cycle.

HSV-1 infection of many tissue culture cells, and some primary cells, causes cytotoxicity(218,219). HSV-1 induced cell death often has characteristics of necrosis and may merely reflect cell lysis resulting from the high viral load within HSV-1 infected cells. However, there is growing evidence that HSV-1 dependent cell lysis is at least partly dependent on cell death signalling pathways, and is not a result of un-programmed necrosis(223). Indeed, HSV-1 has been shown to induce both caspase dependent and independent apoptosis and HSV-1 factors that promote apoptosis have been identified. Although conversely, in the majority of cases, these proteins also have anti-apoptotic effects.

Additionally, there is a growing realization that what would once have been considered uncontrolled cell death, actually results from carefully orchestrated cell signalling events, for example in the cases of pyroptosis and necroptosis(223). As described above, from a morphological perspective, these forms of cell death are indistinguishable from necrosis(138). It seems likely that the cytolytic ability of HSV-1 involves one or more of these pathways, potentially in combination with apoptosis(224). The evidence for this is summarized in Table 5.

A number of HSV-1 genes and cell signalling pathways have been linked with HSV-1 dependent anti-apoptotic and pro-survival signalling, these are summarised in Table 6. Of particular interest is the anti-apoptotic ability of HSV-1 LAT, and the linking of this function with the ability of LAT to re-activate latent HSV-1. Interestingly, many anti-apoptotic proteins can substitute for the LAT as promoters of reactivation(225–228) and pro-apoptotic drugs such as 2[3-(2,3-dichloro-amino]-ethanol (DCPE) and Dexamethasone, can accelerate viral lytic gene expression(221). This was demonstrated in an infection model where latency and reactivation occur within the context of a single replication cycle(221).

It seems probable that during HSV-1 infection multiple cell death pathways, including apoptosis, pyroptosis, and necroptosis, will be both activated and inhibited. The net result of these effects will be dependent upon the stage of the viral replication cycle, the cell type and whether a productive or latent viral infection is established. There are also likely to be autocrine and paracrine effects involved that result as an effect of the HSV-1 induced secretosome, for example those potentially mediated by type I IFN. Considerable opportunities remain to investigate the interaction of HSV-1 with cell death signalling in a clinically relevant cell type such as macrophages.

1.16 HSV-1 infection of macrophages

HSV-1 is an ideal virus with which to study macrophage antiviral strategies. HSV-1 has already been widely used to identify and study PRRs, as described above. Additionally, as an α-herpesvirus, HSV-1 has broad cell tropism (151,229) and has previously been shown to infect human macrophages (197,230–235). Finally, considerable data demonstrate the importance of macrophages during HSV-1 disease, particularly in mouse models of infection, for example in the control of HSV-1 replication at HSV-1 lesions (11–13,236,237). In the absence of macrophages, HSV-1 disease in mouse models of encephalitis, retinitis (11) and keratitis (12) for example, is more severe. In the model of keratitis, in particular, immunopathological corneal destruction following HSV-1 infection is profoundly accelerated by macrophages in the lymph nodes (12).

HSV-1 can infect macrophages *in vitro.* Freshly isolated human monocytes cultured *ex vivo* for a period of at least 3 days become susceptible to productive HSV infection (197,230–235). HSV-1 viral mRNA is expressed (233,235), viral DNA replicated (232), and nascent virions produced (197,232). 80% of a macrophage culture can be infected (231).

Primary monocytes possess a block to HSV replication that is removed upon differentiation into macrophages, for example, differentiation of U937 cells with phorbol 12-myristate 13- acetate (PMA), vitamin D3 or mezerein (a phorbol ester analogue) induces a susceptibility to productive HSV-1 infection (238–241). The mechanism by which this occurs in unknown, although it is known that it is not due to the reduction of nitric oxide upon differentiation (241), and may be due to the dysfunction of ICP0 in undifferentiated cells (240).

Infection of freshly isolated human monocytes with a high MOI of HSV stimulates IFNα production (242–244), even in the absence of viral DNA synthesis (243). HSV-1 infection of macrophages induces type I IFN production, ISG expression and inflammatory cytokine production, including expression of TNFα, CXCL10 (IP-10), CCL2 and CCL3 (191). The type I IFN response is dependent upon Mda5, whereas the production of TNFα, CXCL10, CCL2 and CCL3 is Mda5 independent. Type I IFN has been shown to both inhibit and have no effect on HSV-1 infection (197,232). HSV-1 dependent type I IFN production is also virus strain dependent (197,233,243,245). In these experimental models, the multiple HSV-1 encoded proteins that have been shown to counteract IFN signalling and induction in other contexts, appear to be ineffective during macrophage infection.

HSV-1 can cause cytopathology of primary human monocytes (230,234), U937s (238,246), THP-1s (234,247,248) and mouse peritoneal macrophages (249). HSV-1 dependent death of human monocyte derived macrophages has not been detected (234,235).

In humans, inflammatory macrophages can be detected at HSV reactivation lesions at least 2 days from the first detection of the lesions (250,251) and are thought to play an important role in the early defence against HSV-1 infection (11–13,236,237,252). However, more research is needed regarding the role of macrophages in human HSV-1 infection, for example whether they play a protective role or contribute to the immunopathology that is the hallmark of serious HSV-1-mediated disease. However, given the well documented ability of HSV-1 to infect macrophages *in vitro* and the central role of macrophages to innate immune responses, it is likely that macrophages play an important role in HSV-1 disease especially in the context of HIV-1 co-infection.

1.17 HIV-1

HIV-1, the causative agent of AIDS, was first identified in the the US in 1983(253). Since then, the World Health Organisation (WHO) estimates that over 60 million people have been infected with HIV-1 and that it has been responsible for over 25 million deaths.

HIV-1 belongs to the lentivirus family of retroviruses. Retrovirus virions are enveloped and contain a protein capsid enclosing two copies of the relatively small ssRNA virus genome and a number of viral proteins. One of these proteins is the reverse transcriptase (RT) responsible for reverse-transcribing the RNA genomes into a double-stranded provirus, following virion entry and release of the viral genome from the capsid. The provirus translocates to the nucleus where viral integrase and protease inserts it into the host cell genome, form where it is transcribed. HIV-1 protease also cleaves newly synthesized HIV-1 polyproteins to produce the polypeptides that are assembled into the HIV-1 virion. The HIV-1 replication cycle is represented in Figure (254).

A number of HIV-1 accessory proteins, including Tat and Nef, function to enable LTR transcription and viral gene expression (Table 7). Additionally, the HIV-1 LTR promoter contains specific binding sequences for cellular transcription factors that function to initiate and/or enhance HIV-1 transcription, as reviewed in(255) and summarized in Table 8 (although this list is by no means exhaustive). Given the involvement of cellular factors in the promotion of HIV-1 transcription, it comes as no surprise that co-infecting pathogens, including HSV-1, and cell stimulants can indirectly activate or enhance HIV-1 transcription, and a number of examples of this are described in Table 12. Again, this list is not exhaustive and indeed, it is difficult to find an intracellular pathogen that can *not* increase HIV-1 LTR transcription in some setting. It will be interesting to determine whether HSV-1 interacts with HIV-1 transcription in clinically relevant host cells, such as macrophages. Interestingly, histone deacetylase inhibitors can both enhance HIV-1 transcription and reactivate latent HSV-1(256–259).

1.18 HIV-1 infection of macrophages

Macrophages are major cellular targets of HIV-1 *in vivo* and are thought to contribute to the pathogenesis of disease(9,10). HIV-1 is able to infect macrophages without evident cytopathology, syncytia formation or activation of type I IFN responses *in vitro*(9). This may explain the ability of HIV-1 to establish long-term virus reservoirs in macrophages where it can effectively hide from the adaptive immune responses that deplete HIV-1 infected lymphocytes, although this remains controversial(8).

HIV-1 infection of macrophages and other myeloid cells is sensitive to type I IFN treatment(87,260–262), so the ability of HIV-1 to avoid the innate immune detection mechanisms of these cells may be an important survival strategy for the virus(9,10,87,262). Therefore, understanding the interaction between HIV-1 and macrophages during HIV-1 infection is the subject of active research for those interested in the eradication of HIV-1 and HIV-1 vaccine development(8).

There is controversy over whether HIV-1 directly induces cell death in macrophages(263). There is an extensive body of literature indicating that HIV-1 does not cause cytopathology in macrophages(264). There is also evidence that HIV-1 infection may even protect HIV-1 from apoptosis(265,266). However there have also been reports of HIV-1 induction of apoptosis in macrophages(267,268) and there is evidence that HIV-1 infected macrophages can induce apoptosis of bystander cells(269,270). It will be interesting to see whether any apoptosis or cytopathology observed in herpesvirus infected macrophages is affected by HIV-1 coinfection.

Given the importance of macrophages in HIV-1 infection, it is of interest how co-infecting pathogens will affect the response of macrophages to HIV-1. This may also illuminate aspects of HIV-1 virology, for example, whether HIV-1 infection can protect macrophages from the cell death and type I IFN responses that would otherwise be induced by infection with another virus. Alternatively, whether the type I IFN response induced in response to another virus can protect macrophages from HIV-1 infection.These questions are of particular relevance for opportunistic pathogens that cause disease during AIDS, pathogens that can infect macrophages, pathogens that increase transmission of HIV and pathogens that significantly interact with innate immune responses. HSV-1 satisfies these criteria.

1.19 HIV-1 and type I IFN

Type I IFNs can stimulate expression of antiretroviral genes and restriction factors that inhibit HIV-1 replication and spread *in vitro*. IFNα and β levels in the blood increase significantly following viral dissemination. Despite this, HIV-1 is able to replicate and establish persistent viremia in sero-converted infected patients(113).

Macrophages, monocytes, CD4$^+$ and CD8$^+$ T cells, NK cells and B cells all show evidence of long term stimulation by type I IFNs in HIV-1 infected patients(271–274). Specifically, CD4$^+$ and CD8$^+$ cells from HIV-1 infected individuals have a characteristic pattern of gene expression that suggests chronic stimulation by type I IFNs. Furthermore ISG are induced in the B cells of viremic but not aviremic patients or healthy controls(272). Finally, ISGs are down-regulated in post-HAART lymph-node biopsy samples compared to pre-treatment samples(273).

HIV-1 is able to counteract the antiviral effects of type I IFN observed *in vitro* during pathogenic infection. In fact, it remains to be determined whether the rapid and transient peak of IFNα production in primary HIV-1 infection has a positive or detrimental role in regard to set point determination and progression to AIDS, an effect that is probably also strongly determined by the cells and ISGs that mediate the type I IFN responses. In pathogenic SIV infection of its non-natural host there is a delay between initial infection and IFN production at the transmission site, compared to non-pathogenic infection of the natural host(114). Blocking the IFNR in this model decreases antiviral gene expression, increases the reservoir of target cells and accelerates CD4$^+$ T cell depletion, whereas IFN-α2a administration up-regulates expression of antiviral genes and prevents systemic infection(275). It has also been proposed that one way in which long-term nonprogressors (LTNPs) successfully control HIV-1 infection is by a type I IFN dependent containment of HIV-1 at the transmission sites(116), allowing enough time for the adaptive immune system to mount an efficient response to the virus prior to virus dissemination. However, long term stimulation by type I IFN has been proposed as a mechanism for the aberrations of immune cell function characteristic of chronic HIV-1 disease. In the pathogenic SIV infection model, continued IFN-α2a treatment induces type I IFN desensitization and decreases antiviral gene expression. This leads to an increased SIV infection reservoir size and accelerates CD4$^+$ T-cell loss. It is therefore clear that type I IFNs play some role in AIDS but it remains to be determined if they are beneficial or detrimental to the host.

Production of type I IFN by HIV-1 infected cells can promote apoptosis of uninfected bystander CD4$^+$ T cells and up-regulate cell-cycle control associated ISGs, leading to the lymphopenia that is the hallmark of AIDS(276,277) . IFNβ, the main type I IFN produced during initial immune responses to the presence of HIV-1 in the central nervous system (CNS), is thought to be linked to progression to AIDS associated dementia(278). On the other hand a recent study by Tavel *et al* showed that treatment of asymptomatic HIV-1 positive patients with IFNα-2b can significantly decrease HIV-1 RNA levels in the blood, without reducing the percentage of CD4$^+$ cells present(279) .

1.20 SAMHD1 and vpx

In this study I use a single-round model of HIV-1 infection where envelope (env) deleted HIV-1 (ΔenvHIV-1) is VSV-pseudo-typed and delivered to macrophages *in trans* with VSV-SIV expressing vpx. Vpx is an HIV-2 and SIV accessory protein packaged into viral particles via an interaction with Gag(280–282). It is necessary for the efficient replication of HIV-2 and SIV in myeloid cells(283–287). Vpx also enhances HIV-1 infection of macrophages(288), enabling the study of macrophage responses to single-round HIV-1 infection.

Single-round infection models and lentivector systems have demonstrated that vpx plays a role in the nuclear import of pre-integration complexes in non dividing cells such as macrophages(289,290). Upon virion entry, vpx localises to the nucleus of infected cells(290–292). It is controversial as to whether vpx then shuttles between the cytoplasm and nucleus, and whether this underlies the function of vpx in nuclear import(292).

Vpx increases the efficiency of HIV-1 infection of macrophages by degrading the cellular restriction factor SAMHD1(293,294). SAMHD1 has broad activity against a range of retroviruses(295,296) as well as DNA viruses such as VZV and HSV-1(297,298). It is a deoxynucleoside-triphosphate (dNTP) phosphohydrolase(299–301) that decreases the pool of dNTPs available for reverse transcription in myeloid cells and non-cycling T cells(302–304). The enzymatic and restriction activity of SAMHD1 is inhibited by CDK1 mediated phosphorylation(305,306). CDK1 is inactive in resting cells, and given that SAMHD1 levels are equivalent in active T cells and macrophages(304), this, along with the higher dNTP concentration in cycling T cells than macrophages(307), may explain the inability of SAMHD1 to restrict HIV-1 replication in cycling T cells. SAMHD1 may also mediate a dNTP independent restriction effect, for example it can interact with and degrade specific nucleic acids, including ssRNA and ssDNA(308,309). Vpx recruits the DCAF1-DDB1-CUL4A E3 ligase complex(310–313), in order to facilitate degradation of SAMHD1. The interaction between SAMHD1, vpx and HIV-1 are represented schematically in Figure 8.

The susceptibility of HIV-1 infection of macrophages to type I IFN mediated restriction(87,260–262) has been partly attributed to the activity of SAMHD1, not least because the IFN induced block is primarily exerted at the level of DNA accumulation(87,261,262), and vpx increases HIV-1 replication in IFN-treated myeloid cells(314–316). Although not an interferon inducible gene(316), SAMHD1 is dephosphorylated, and thereby enzymatically activated, by type I IFN stimulation(305). Furthermore, IFN mediated restriction of HIV-1 replication can be reduced, if not abolished, by delivery of vpx or silencing of SAMHD1(316). Recently, HSV-1 has been shown to counter-act SAMHD1 activity(298). It will be interesting to see whether vpx interacts with HSV-1 infection of macrophages.

Vpx also sequesters and decreases the stability of APOBEC3A(317,318), an ISG and potent restriction factor of HIV-1 replication in myeloid cells(319–321).

1.21 HSV-1 and HIV-1 co-infection

HSV-1 and HIV-1 interact at an epidemiological, clinical and molecular level. An estimated two thirds of the individuals infected with HIV-1 worldwide are also co-infected with HSV-1/2(322,323). HIV-1 and HSV have similar routes of infection, can infect the same target cells, interact significantly with the immune system and have high prevalences within the same regions of Sub-Saharan Africa(176). At the population level, HSV infection is correlated with increased HIV-1 transmission and disease severity(14,15,104,324–338) and *vice versa*(176,323,339–342). Additionally, HIV-1 infection allows re-activation of latent HSV-1 via suppression(343) and promotes HSV-1 dependent life-threatening disease during AIDS(14).

HSV infection increases HIV-1 transmission within a population(14,15,104,325,330–333). In a meta-analysis Freeman *et al* demonstrated that HSV-2 sero-positivity was a statistically significant risk factor for HIV-1 acquisition in general populations of men (summary adjusted risk ratio [RR] 2.7; 95% Confidence Interval [CI] 1.9-3.9) and women (RR, 3.1; 95% CI 1.7-5.6). Furthermore, a recent mathematical model describing the transmission dynamics of HIV-1 and HSV, demonstrated that HSV infection may not only increase the risk of HIV-1 transmission but also may increase the peak of HIV prevalence, as well as decreasing the time to that peak(344).

The increased susceptibility of HSV infected individuals to HIV-1 infection, and/or the increased infectivity of HSV/HIV-1 co-infected individuals, may account for the population level interactions between HSV infection and HIV-1 transmission(104,327,328,345–349). Both HSV-1 and HSV-2 cause genital ulceration and mucosal disruption, and thereby provide a portal of entry to HIV-1(329). Indeed, the infectiousness of individuals positive for HIV-1 increases during periods of HSV reactivation(350), and HSV genital reactivation, including asymptomatic shedding, increases the concentration of HIV-1 in plasma and genital secretions(327,328,345,351). Furthermore, HIV-1 target cells, including $CD4^+$ lymphocytes and macrophages, are recruited to herpetic lesions(104,335,352,353). In a study of the heterosexual transmission between HIV-1 discordant couples in Uganda, genital ulcers resulting from HSV-2 infection was associated with a four fold increase in HIV-1 transmission(325).

HIV-1 is correlated with increased HSV-1 infectivity(14). A number of mechanisms may be responsible for this, for example, HIV-1/HSV-1 co-infection increases HSV shedding, HSV reactivation rates and the duration of hepatic ulcers(176,340,350,354–357). Likely to be of particular importance is the immunosynergistic crosstalk between HIV-1 and HSV. HIV-1 co-infection impairs HSV specific $CD4^+$ and $CD8^+$ T cell responses(356,357), and decreases Th1 cytokine and CCR5 ligand secretion in response to HSV infection(343). Additionally, HIV-1 can facilitate HSV-1 entry by disrupting the oral mucosal epithelial(358). The HIV-1 accessory proteins Tat and Gp120 disrupt epithelial tight junctions(359–361), whilst HIV-1 infection also disrupts adhesion junctions by changing the localisation of the cell surface molecule, nectin-1(358,362–364). Nectin-1 binds gD and is responsible for facilitating HSV-1 entry into epithelial cells and HSV-1 cell-to-cell spread. Under normal circumstances, nectin-1 is sequestered in intracellular junctions, limiting the access of HSV gD to this receptor(365). HIV-1 disrupts normal nectin-1 localisation and increases its availability to HSV-1.

Untreated AIDS patients can demonstrate severe HSV-1 disease, as a result of being immunocompromised. Additionally, HIV-1 enhances the severity of HSV-1 associated disease(366).

Transfection of HSV-1 specific genes can activate HIV-1 transcription, for example the HSV-1 IE protein ICP0 upregulates HIV-1 LTR transcription by recruiting NF-κB to the LTR promoter(335,367,368). HIV-1 and HSV coinfection of CD4$^+$ cells(369–371) and DC(352,372) has been demonstrated. HIV-1/HSV-1 co-infection of macrophages has not been investigated, although HSV-2 has been shown to increase the susceptibility of macrophages to HIV-1 infection by upregulating CCR5 expression levels on the cell surface(373) and NF-κB activation by heat-inactivated HSV-1 virions can induce HIV-1 replication in macrophages(374). However, another study has shown that HSV superinfection of PBMC from HIV-1 infected individuals, leads to greater HSV-1 virion production than infection of PBMC from healthy donors, but that HSV-1 super-infection decreases HIV production(375). On the other hand, HSV has been shown to increase expression of $\alpha_4\beta_7$ and thereby facilitate amplification of HIV target cells. The mechanism by which this occurs is unknown.

1.22 Summary

Considerable opportunity exists to explore the potential interactions between HSV-1, HIV-1 and macrophages. Despite the clear potential relevance of macrophages to HSV-1 infection *in vivo*, current knowledge is lacking in regard to the character of HSV-1 infection in these cells. There is also considerable opportunity to explore the interactions of HSV-1 with macrophage cell death and type I IFN responses. HIV-1 infection of macrophages is currently the subject of intense interest in the field. Studying HIV-1 infection in the context of HSV-1 co-infection may provide novel insights into how both these viruses interact with this important cell.

1.23 Objectives

In the following chapters, I will attempt to establish the following:

1. To confirm that HSV-1 can infect macrophages, and to investigate whether the infection is productive and/or lytic and whether it induces a type I IFN response. I will also investigate whether a latent HSV-1 infection of macrophages can be established.
2. To investigate the virological and cellular consequences of HIV-1 and HSV-1 co-infection of macrophages.

2 Methods

2.1 Macrophages

The macrophages used in this study were derived from adherent monocytes that were isolated from the peripheral blood of volunteers, and differentiated into macrophages using recombinant M-CSF (GIBCO, Cat. No. PHC2044) as previously described (9) (376). The study was approved by the joint University College London/University College London Hospitals National Health Service Trust Human Research Ethics Committee and written informed consent was obtained from all participants.

Adherent monocytes were differentiated into macrophages using RPMI 1640 (GIBCO Invitrogen) with 10% autologous heat-inactivated human serum (HS) and 20 ng/mL M-CSF (R& D systems) for 3 days (Fig.9). The monocytes were allowed to adhere to the bottom of the wells of either plastic 24 cell plates or CellCarrier-96 well black, optically clear bottom 96 well plates (product code 6005558). The cells were incubated at 37°Cin a 5% CO_2 atmosphere.

On day three, non-adherent cells were removed by repeated washing. After washing, the medium was replaced with RPMI 1640 containing 5% type AB human serum (Sigma-Aldrich). HSV-1 infections or HIV-1 infections were performed 6 days after isolation of the cells from peripheral blood. This model of monocyte derived macrophages has been extensively optimized and has been shown to have negligent T cell contamination by day 6 of the protocol (377).

For each experiment, at least 3 replicates were performed with cells derived from different donors. The approximate macrophage number per well could be estimated by determining the total number PBMC isolated from every donor and assuming that adherent monocytes to comprise 5% of these cells.

2.2 Cell lines

Vero (African green monkey, immortalised fibroblast-like kidney cells), HeLa (Human, immortalised cervical cancer cells) and HEK293T (Human, immortalised embryonic kidney cells) were cultured in Dulbecco's modified Eagle's medium (DMEM) media supplemented with 10% fetal calf serum (FCS). They were grown as monolayers at 37°Cin a 5% CO_2 atmosphere or 10% CO_2 atmosphere for the HEK293T cells. All cells were passaged by trypsination and no more than 30 times.

2.3 HSV-1

Three strains of HSV-1 were used; SC16, +17-gB-GFP and +17-ICP0-YFP. These were kind gifts from Dr Richard Milne (SC16) or Dr Melori Jones (+17-gB-GFP and +17-ICP0-YFP).

HSV-1 was propagated in Vero cells. The supernatants were harvested when $\leq$90% cytopathic effect was observed in vero cells in 175cm^2 tissue culture flasks infected with HSV-1 at a MOI of 0.01pfu/cell. The supernatants were centrifuged at 3000xg for 10 minutes, passed through a 0.45 filter and ultracentrifuged through a 25% sorbitol cushion for 2 hours at 23,000rpm at 4°Cusing a SW28 rotor. The resulting pellet was resupended in RPMI 1640 (GIBCO Invitrogen) and stored at -80°C. The virus was not passaged more than 4 times.

Purified HSV-1 and the supernatents from HSV-1 infected macrophages were titrated by plaque assay on vero cells, as has been previously described. Vero cell monolayers established in 24 well plates.

HSV-1 virus was serially diluted in DMEM then each dilution used to infect a well of vero cells. After 24 hours, the media was removed, and replaced with DMEM-carboxymethylcellulose (2:1) media, and the cells incubated as normal. 3 days post infection the media was removed, the cells fixed with ice cold methanol and stained. The number of plaques were counted in the wells with a number of plaques in the range of 5-20, and the concentration of infectious HSV-1 particles per ml of the original inoculum calculated. A virus titre in the range of $1\text{x}10^8$-10^9 was generally achieved.

2.4 Lentiviruses

Vesicular stomatitis virus (VSV)-G envelope pseudotyped replication deficient HIV-1 (R9 BaL Δenv), full-length HIV-1 (R9 BaL) and VSV-pseudotype vpx were produced by transfecting HEK293T cells with the appropriate plasmids (Table 9). The day before transfection, $6{,}5\text{x}10^5$ HEK293T cells, passaged in culture for at least 1 week, were seeded onto a 100mm tissue culture dish. 4 hours before transfection, the media was refreshed. The cells were transfected using FuGENE 6 (Cat. No. E2691/2/3). For the transfection of one plate of cells 18μl of FuGENE 6 was mixed with 200μl Opti-MEM®reduced serum media and 4.5μg of the DNA plasmid to be transfected. The transfection mixture was incubated at room temperature for 20 minutes then added to the cells in a dropwise manner. After 24 hours the media was refreshed. For the next 3-4 days the supernatent was collected daily, until the transfected cells had lost viability and detached.

The supernatants were centrifuged at 3000xg for 10 minutes, passed through a 0.45 filter and ultra-centrifuged through a 20% sorbitol cushion for 2 hours at 23,000rpm at 4°Cusing a SW28 rotor. The resulting pellet was resupended in RPMI 1640 (GIBCO Invitrogen) and stored at -80°C. All HIV-1 viruses were titrated by LTR qPCR. The virus was lysed using RLT buffer, cDNA synthesised and RT-qPCR performed as described below. The HIV-1 LTR copies/ml were determined using a standard curve generated from qPCR during the same run, of a serially diluted virus sample of known copy number. SIV3+ virus was titrated by RT-ELISA (Roche, Product code: 11468120910).

2.5 UV inactivation of HSV-1 and HIV-1

HSV-1 or HIV-1 was inactivated by exposure to UV light, at a distance from the light of 10cm, for 10 minutes, in a sterile environment. UV-inactivation of HSV-1 and HIV-1 were confirmed by plaque assay on vero cells or LTR qPCR following macrophage infection, for HSV-1 and HIV-1 respectively.

2.6 Macrophage infections

6 days post isolation, or 2 weeks following HSV-1 infection, macrophages were infected with HIV-1 diluted to various concentration in RPMI, in the presence of 3ng/ml of SIV3^+ (vpx). The inoculation volume was 500μl and the media was replaced with RPMI + AB serum 6 hours after infection. Macrophages were infected with HSV-1 either 6 days post isolation, or 6 days post infection with various concentrations of HIV-1 in the presence of vpx. The estimated dose of HSV-1 for each infection was calculated as the plaque forming units of HSV-1, as titred on vero cells, per macrophage (pfu_{vero}/cell). Macrophages were inoculated with the desired dose per macrophage of HSV-1, in a volume of 200μl or 500μl of RPMI supplemented with 10% FCS, for 96 well or 24 well plates respectively. 1 hour post inoculation, the inoculation media was replaced with RPMI supplemented with 10% FCS. During the establishment of a latent HSV-1 infection, the media was replaced every 3 days.

2.7 Viability

Cell viablity was assessed with the alamarBlue assay (AbD Serotec, Cat. No. BUF012B)(378). Macrophages or 293T cells in a 24 well plate were washed twice with PBS and the media replaced with with 500μl of RPMI suplemented with 5% AB serum and 10% alamarBlue®. The cells were incubated at 37°CC for 3 hours. 100μl of the media was collected from each well simultaneously, placed in individual wells of a flat bottom 96 well plate and the relatively absorbency of the media at 570nm and 595nm measured in a spectrometer. These values were used to calculate the percentage of alamarBlue that had been reduced by the cells, a value linearly correlated with the number of viable cells in each well. First, the correction factor R_0 was calculated:

$$R_0 = \frac{AO_{LW}}{AO_{HW}}$$

Where:

AO_{LW} = Absorbance of AB in media - Absorbance of media only

AO_{HW} = Absorbance of AB in media - Absorbance of media only

Which can then be used to determine the percentage difference between treated and control cells:

$$X = \frac{TA_{LW} - (TA_{HW} \times R_0)}{CA_{LW} - (CA_{HW} \times R_0)} \times 100$$

Where:

X = Percentage difference between treated and control cells

TA_{LW} = Absorbance at lower wavelength (i.e. 570nm), test well, minus the media blank

CA_{LW} = Absorbance at lower wavelength (i.e. 570nm), control well, minus the media blank

TA_{HW} = Absorbance at higher (i.e. 595nm) wavelength, test well, minus the media blank

CA_{HW} = Absorbance at higher (i.e. 595nm) wavelength, control well, minus the media blank

R_0 = Correction factor

2.8 Lysis

Cell lysis was assessed by LDH ELISA. The supernatents were collected from macrophages either prior to infection at various time points post infection with HSV-1, and assessed for the presence of LDH by ELISA. The concentration of LDH per sample was expressed as a percentage of the concentration of LDH in the supernatant of a sample collected following complete cell lysis resulting from the addition of a lysis buffer. In this way, the percentage of cell lysis in the HSV-1 infected macrophages was calculated.

2.9 Nuclei counting and morphology

HSV-1 infected or mock infected macrophages in a 96 well plate were fixed with 4% PFA. The nuclei were stained with DAPI (1:2400) (Cell Signalling Technology®, Cat. No. 4083) for 5 minutes and the of cells plate imaged using a high-throughput immuno-fluorescent microscope. Metamorph image analysis software was used to count the number of nuclei per well of the plate, as well as the average intensity and area of each nuclei. These data were converted from txt files into fcs files(379), and analysed using flowJo.

2.10 FACS

To assess plasma membrane integrity of HSV-1 infected cells, macrophages were mock infected or infected with HSV-1-ICP0-YFP and detached from the tissue culture plates 6hpi by trypsinisation for 45 minutes, following which they were incubated with florescent labelled Annexin-V before FACS.

2.11 RT-qPCR

HSV-1 infected, HSV-1/HIV-1 co-infected or mock infected macropahges were lysed and the Qiagen RNAeasy plus kit (Cat. No. 74136) used to extract and purify RNA from the cell lysates. The RNA was DNase treated (Life Technologies, Turbo DNA-free kit, Cat. No. AM1907) to remove contaminating DNA from the sample. cDNA was synthesized from the RNA using the qScript cDNASupermix kit (Quanta BioSciences, Cat. No. 95047-025) and quantitative (q)PCR for selected genes performed using ABI Prism 7000 (Applied Biosystems) and specifically designed primers and probes (Sigma-Aldrich), in the case of HSV-gB, GAPDH, IP10, or TaqMan inventoried assays (IFI16 (Hs00194261 m1), RIG-I (Hs00204833 m1) and LTR (Pa03453409 s1) using ABI Prism 7000 (Applied Biosystems). Transcript levels were quantified by the 2-$\Delta\Delta C_{+}$ method and normalization to GAPDH expression.

2.12 Immuno-florescence

HSV-1 infected, HSV-1/HIV-1 co-infected or mock infected macropahges in a 96 well plate were fixed with 4% PFA, blocked and permeabilised with 10% goat serum/Triton 100x for 30mins, then incubated at 4°CC overnight with primary antibody. The cells were washed three times with PBS, and incubated for 1 hour with the secondary antibodies PBS supplemented with 10% goats serum. The nuclei were stained with DAPI (1:2400) (Cell Signalling Technology®, Cat. No. 4083) for 5 minutes and the of cells plate imaged using a high-throughput immuno-fluorescent microscope. Immuno-flourscent images were analysed using ImageJ/Fiji and Metamorph image analysis software. Data processing was done using R scripting and flowJo. The primary antibodies used were mouse anti-ICP4 (ab6514, 1:1000) and mouse anti-ICP0 (ab6513, diluted 1:10,000). The secondary antibodies used were Alexa555 anti-mouse (1:1000) and Alexa488 anti-rabbit (1:1000)

2.13 Virion release

To assess HIV-1 virion release, the supernatents were collected from HSV-1 infected, HSV-1/HIV-1 co-infected or mock infected macropahges, and the cell-free HIV-1 gag (p24) concentrations quantified by ELISA with recombinant standards (kit version 9.6, from AIDS Vaccine Programme, National Cancer Institute- Fredrick). Intracellular detection of p24 was performed as previously described(9,376).

To assess HSV-1 virion release, the media was removed from HSV-1 infected, HSV-1/HIV-1 co-infected or mock infected macrophages, the cells washed 3 times with PBS and replaced with fresh media. After 24 hours the supernatents were and assessed from the presence of infections HSV-1 virion by plaque assay on vero cells.

2.14 Western blot

Macrophages in 24 well plates were lysed using SDS sample buffer. The samples were boiled for 5 minutes then passed through a needle. The homogenised samples were then loaded into a NuPAGE 12% gels, 1.0mm width (Cat. No. NP0343BOX), immersed in NuPAGE sample buffer along with a protein ladder (BioLabs, Cat No. P7711S). The samples were run for 30 minutes at a 100V. The proteins were then transferred from the onto a nitrocellulose membrane immersed in NuPAGE transfer buffer. The membranes were washed, blocked overnight at 4°Cin milk then stained for ICP4 or actin.

2.15 Blocking the type I IFN receptor

Macrophages were incubated in media containing a mouse monocolonal antibody against human IFNα/β receptor chain 2 (MMHAR-2) (PBL Interferon Source, Cat. No. 21385-1). After 1 hour the macrophages were stimulated with recombinant type I IFN or infected with HSV-1, in the presence of the blocking antibody.

2.16 Detection of caspase activity

Macrophages or HeLa cells were infected with HSV-1 or stimulated with staurosporine. After 6 or 24 hours the macrophages were incubated in media containing 10μM CaspACE FITC-VAD-FMK *in situ* marker (Promega, Cat. No. Promega) for 30 minutes. The cells were then fixed, stained with DAPI and imaged.

3 HSV-1 infection of macrophages

3.1 Introduction

In this chapter, I characterize HSV-1 infection of human macrophages, focusing on whether or not HSV-1 can establish a productive, lytic infection and the nature of the host cell response to the infection.

As important cells of the innate immune system(5–7), macrophages are particularly relevant to the study of host pathogen interactions. HSV-1 is widely used to elucidate general principles of pathogen sensing and innate immune responses(190), and readily infects macrophages(197,230–235). HSV-1 therefore provides an ideal tool with which to investigate host-pathogen interactions within macrophages. Additionally, macrophages are known to play a role in HSV-1 disease(11–13). Consequently, understanding the HSV-1 and macrophage interaction will provide insights into both macrophage immunology and HSV-1 virology relevant to *in vivo* infection.

Whilst the ability of HSV-1 to infect macrophages has long been established, there remains considerable disagreement regarding whether or not the infection is lytic, productive and/or induces a type I IFN response. I address these questions using the following experimental approaches:

First, I use the SC16 strain of HSV-1 which was originally isolated from an oral HSV-1 lesion(380). Previous investigations of HSV-1 infection of macrophages have used a range of laboratory strains. HSV-1 is less prone to genetic mutation *in vitro* than other herpesviruses, but there are still significant differences between clinical and laboratory strains(154,187). SC16 has a greater ability to cause pathogencity in a range of HSV-1 disease models compared to other widely used strains(381) and is therefore more clinically relevant.

Second, I assess the expression of a range of HSV-1 genes in order to investigate infection within my model. HSV-1 infection of macrophages has been previously demonstrated at both the transcript and protein level(233,235). However, rarely has more than one gene or protein been assessed in an individual study. I evaluated expression of the IE proteins ICP0 and ICP4, the early gene DNA Pol, the leaky late gene gB and the late and latency gene LAT. The protein products of these genes are multifunctional. gB, for example, is a fusion protein that primarily functions to facilitate entry of the virion(382–386) but which also contributes to HSV-1 immune evasion(387,388) (Table 10). ICP0 is an IE protein that facilitates HSV-1 gene expression and interacts with the host immune system(389). It is not necessary for infection but plays a key role in the establishment of and reactivation from latency(390–393) (Table 11). ICP4 is another IE protein involved in transcription tranactivation. DNA pol is the enzyme responsible for replicating the viral genome. LAT is the only gene product expressed during latency and may have a role in promoting cell survival and facilitating reactivation from latency. Assessing the expression of a range of HSV-1 genes, expressed at different time points in the viral replication cycle, should establish if and how HSV-1 replication progresses in macrophages. In particular, expression of ICP0, ICP4, gB, DNA Pol and LAT were chosen as their expression can be used as markers of different stages of the HSV-1 replication cycle. I also assess *de novo* production of infectious virions. Virion production by HSV-1 infected macrophages has only been demonstrated by a few groups(197) and remains controversial(394).

Third, I measure the type I IFN responses in HSV-1 infected macrophages by quantifying the expression of a number of ISGs. In previous reports of HSV-1 infection of macrophages, the type I IFN response has been investigated by measuring IFNα production and *ifnb* transcription(191,197,242–244,395), an approach that has produced conflicting data. This is potentially due to cytokines from the cells used to produce the virus contaminating the virus stocks, in particular macrophages are very sensitive to IFN-β. Another explanation is the transient nature of *ifnb* gene up-regulation. Therefore, to reduce the risk of contamination with cellular cytokines, I produce HSV-1 in vero cells, a well characterized kidney epithelial cell line originally extracted from the African green monkey in 1962, and used to produce a wide variety of viruses and vaccines(396). Vero cells lack the capacity to produce type I IFN(397). I also purify the virus by passing it through a sorbitol cushion, as has been previously demonstrated(398). Additionally, I assess the transcript levels of a range of ISGs, including IP10, IFI16, RIG-I and IPS-1, over time. IP10 up-regulation is known to be particularly stable following IFN-β stimulation(9).

Finally, I quantify cell death by a variety of methods, including assessment of the viability, lysis, nuclear count and membrane permeability of macrophage cultures infected with HSV-1. The mechanisms by which HSV-1 induces cell lysis have been relatively neglected by investigators. There is no consistency in the literature even regarding whether or not HSV-1 causes apoptosis in tissue culture cells such as HeLa cells, let alone macrophages(399,400). Therefore, I attempt to establish the mechanism of cell death in HSV-1 infected macrophages by using inhibitors of specific cell death pathways.

The objectives of this chapter are as follows:

1. To establish if macrophages can be productively infected with HSV-1 by assessing IE, E and L gene and protein expression, virion production and cell survival.
2. To investigate the mechanisms by which HSV-1 may induce macrophage death.
3. To establish if HSV-1 infection up-regulates type I IFN in macrophages, and to begin to investigate the mechanisms by which this may occur.

3.2 Results

3.2.1 HSV-1 infects macrophages

Human monocyte derived macrophages, differentiated from adherent PBMC using M-CSF (Fig.9), were exposed to HSV-1 and assessed for HSV-1 gene and protein expression (Fig.10). The IE proteins ICP0 and ICP4 were expressed within 6 hours, as shown by immuno-florescent staining (Fig.11a and Fig.11b), western blot (Fig.12a) and FACS (Fig.12b [1-way ANOVA, $p \leq 0.0001$]). The percentage of macrophages positive for HSV-1 IE protein was dose dependent and saturated at approximately 50-60% (Fig.12c [1-way ANOVA, $p \leq 0.0001$] and Fig.12d [1-way ANOVA, $p \leq 0.0001$]).

HSV-1 infected macrophages also expressed HSV-1 early and late genes. gB transcript and protein was dose dependent and observed within 6 hours of infection (Fig.13a, Fig.13b [1-way ANOVA, $p \leq 0.0001$], Fig.13d and Fig.13e [1-way ANOVA, $p \leq 0.0001$]). The expression of the cellular housekeeping gene GAPDH was unaffected, except following infection with a very high MOI (Fig.13c [t-test, $0.01 < p \leq 0.05$]). gB and HSV-1 DNA Pol expression were detectable as early as 2hpi and increased in a time dependent fashion (Fig.13e [2-way ANOVA, $p = 0.0004$], Fig.14a [2-way ANOVA, $p = 0.0389$]). LAT expression did not increase in a time or dependent manner (Fig.14b [2-way ANOVA, $p = 0.4329$]).

HSV-1 gene expression has previously been shown to be dependent on NF-κB activation(401). NF-κB was activated within HSV-1 infected macrophages in a HSV-1 dose dependent manner (Fig.14c [2-way ANOVA, p$\leq$0.0001]). NF-$kappa$B expression could be inhibited in either HSV-1 infected or LPS stimulated macrophages using a specific small molecule inhibitor (Fig.14c [2-way ANOVA, p$\leq$0.0001]). Inhibiting NF-κB decreased HSV-1 E and L gene expression (Fig.14d). HSV-1 late gene expression could be eliminated by coating the HSV-1 virions with soluble heparin prior to inoculation of the macrophage cultures, so as to prevent virion entry (Fig.15a and Fig.15b)(402,403). HSV-1 late gene expression was also sensitive to acyclovir (Fig.15c [2-way ANOVA, p$\leq$0.0001]). Importantly, I confirmed that the RNA used for qPCR was not contaminated with viral DNA (Fig.15d).

3.2.2 HSV-1 infection induces a type I IFN response

IP10 is widely recognised as an ISG. I confirmed that IP10 is up-regulated in macrophages in response to stimulation with type I IFN, DNA or RNA (Fig.16a). HSV-1 infection of macrophages up-regulated IP10 in both a dose and time dependent fashion as compared to the effect of UV-HSV-1 stimulation (Fig.16b [2-way ANOVA, p$\leq$0.0001]). IP10 up-regulation in response to both IFN-β and HSV-1 infection was dependent on engagement of the IFNR (Fig.16c [2-way ANOVA, p$\leq$0.0001] and 16d [2-way ANOVA, p$\leq$0.0001]). HSV-1 infection of macrophages also up-regulated the ISGs IFI16, Mda5 and IFN-β (Fig.16e). RIG-I up-regulation was not significant. Inhibiting NF-κB inhibited HSV-1 dependent up-regulation of IP10 (Fig.16f [2-way ANOVA, p$\leq$0.0249]).

3.2.3 HSV-1 productively infects macrophages

HSV-1 productive infection of epithelial cells results in virion release and cell death(151). I investigated whether this was also the case for HSV-1 infection of macrophages. I assessed cell death in HSV-1 infected macrophages by three methods: measuring cell metabolic activity with the alamarBlue assay, determining cell lysis by LDH ELISA and using nuclear counting as a measure of cell number (Fig.17a).

The alamarBlue assay an assay of metabolic activity that assesses cell death by exploiting the fact that in a population of cells cultured in media containing alamarBlue, the rate of alamarBlue reduction is linearly related with the number of viable cells within a cell population. This is the case within a range of approximately 4 orders of magnitude, as demonstrated with HEK293T cells (Fig.18a). As measured by this assay, macrophages infected with HSV-1 lost viability by 48hpi at high but not low MOIs (Fig.18b). LDH release, used as a measure of cell lysis, was also observed 48hpi at high MOIs (Fig.18c). Death did not occur following exposure of macrophages to UV-HSV-1 (Fig.18b and Fig.18c) and was sensitive to acyclovir (Fig.18e and Fig.18f). HSV-1 infection of macrophages resulted in production of viable virions, as early as 24hpi and at both medium and high MOIs, in a time dependent manner (Fig.18d [1-way ANOVA, p=0.00635]). Nuclei counting is a more sensitive measure of cell death than the alamarBlue or LDH assay. Cell number as determined by this method was reduced within 6 hours of infection with HSV-1. This was HSV-1 dose dependent and occurred following even medium doses of HSV-1 (i.e. at an MOI of 10) (Fig.19a). HSV-1 dependent cell death assessed by this measure was also dose dependent (Fig.19a [1-way ANOVA, p=0.0059]) and sensitive to acyclovir (Fig.19b), indicating that replicative infection was necessary for this effect.

3.2.4 The membrane permeability of HSV-1 infected macrophages

Activation of cell death pathways can be detected by assessing the plasma membrane integrity of cells by flow cytometric quantification of Annexin-V(404). Annexin-V stains cells that are necrotic, pyroptotic or apoptotic. Surprisingly, up to 30% of macrophages exposed to UV-inactivated HSV-1 encoding YFP tagged ICP0 (HSV-1-ICP0-YFP) were positive for Annexin-V staining (Fig.20a [2-way ANOVA, p=0.0001]), despite the fact that UV-HSV-1 does not reduce macrophage viability (Fig.18b), cause cell lysis (Fig.18c) or reduce nuclear count (Fig.19a). There was no significant effect of UV-inactivation on the effect of the HSV-1 dependent increase in Annexin-V positivity (Fig.20a [2-way ANOVA, p=0.1671]). At face value, these data suggest that at 6hpi, macrophages infected with replication competent HSV-1 are neither apoptotic or necrotic, given that they appear to have intact membranes and do not increase expression of Annexin-V on their outer membrane. However, detaching macrophages from tissue culture plastic for FACS analysis is technically difficult and the data may be confounded by the increased membrane permeability of the macrophages resulting from prolonged trypsinization.

3.2.5 HSV-1 induces caspase activation

To determine if HSV-1 dependent macrophage death involves a programmed cell death pathway, HSV-1 infected macrophages were assessed for pan-caspase activation using a fluorescent intracellular indicator of pan-caspase activity. The indicator detected caspase activation in a well-established model of apoptosis, staurosporine stimulated HeLa cells, but not mock-stimulated cells (Fig.20b). Caspase activation was also observed in both HSV-1 infected HeLa cells and macrophages (Fig.20c). The percentage of cells demonstrating caspase-activation was highest 6hpi, at a medium MOI (Fig.20d).

3.2.6 Using nuclear morphology to assess cell death

Apoptotic cells possess a unique nuclear morphology that distinguishes them from cells dying by other programmed cell death pathways, such as pyroptosis and necroptosis. High-throughput microscopy can give an analysis of nuclear morphology considerable power. As proof of this concept, the nuclear morphologies of HeLa cells exposed to different conditions were analysed. HeLa cells were infected with HSV-1 or incubated with either staurosporine or a DMSO loading control, and then stained with DAPI and imaged. Staurosporine treated HeLa had a classic apoptotic nuclear morphology, with condensed, small, bright nuclei and apoptotic bodies (Fig.20b). HSV-1 infected HeLa had nuclei with opposite morphology, they were larger and dimmer than the DMSO control (Fig.20b).The DAPI stained nuclei were individually scored for average intensity, area, integrated intensity (total intensity) and average intensity/area ratio. A 2D density plot of the average intensity and nuclear area of all nuclei (n$\sim$200,000) demonstrated the existence of distinct populations of nuclei within even a mock infected population (Fig.21a), representing different stages of cell division. The scales in this plot are arbitrary.

An apoptotic population would be expected to have nuclei with a higher average intensity/area ratio than a non-apoptotic population. Indeed, the nuclei of cells within a population of HeLa cells incubated with staurosporine, had either the same average intensity/area ratio value as control HeLa cells or a high "apoptotic" average intensity/area ratio (Fig.21b). In line with not being apoptotic, HSV-1 infection decreased the average intensity and increased the area of the HeLa nuclei. Consequently, the probability density of the average intensity/area ratio for these cells was shifted to the left compared to the DMSO control. HSV-1 infected HeLa are therefore not apoptotic and have nuclei with a more necrotic-like morphology.

There were also multiple nuclear morphology types within a macrophage population exposed to HSV-1 (Fig.21d). At 24hpi, a scatter-plot of nuclear area and nuclear ICP4 average intensities of all the macrophages in an experiment (including unstained control cells) revealed 3 distinct populations of cells (Fig.21c):(1) the unstained control population, (2) an ICP4$^-$ population with a background level of non-specific staining and (3) an ICP4$^+$ population of cells. The cells exposed to HSV-1 are shown in blue. Furthermore, a 1D density plot of nuclear intensity over nuclear area revealed that at a high MOI a greater proportion of macrophages have small, dense nuclear compared to mock infected, and at low MOI (1-10pfu$_{vero}$/cell) (Fig.22a). Furthermore, the plot shows that a population of macrophages exposed to HSV-1 had a larger and less dense nuclei morphology than macrophages exposed to UV-HSV-1 (Fig.22a), indicating that at low MOI macrophages infected with HSV-1 are not apoptotic. This is shown more clearly by plotting the percentage of nuclei within the upper left or lower right quadrants of the scatter plot (Fig.22b [2-way ANOVA p=0.0007] and Fig.22c).

3.2.7 The mechanism(s) of HSV-1 dependent macrophage death

Small-molecule inhibitors of apoptosis, pyroptosis and necroptosis were assessed for their ability to prevent HSV-1 dependent cell death (Fig.23a). 48hpi, none of the inhibitors protected macrophages at high MOIs of HSV-1 infection (Fig.23b) and all of the inhibitors significantly protected macrophages at a low MOI (1pfu$_{vero}$/cell). In between these extremes, only the necroptosis inhibitor completely protected macrophages from HSV-1 dependent death, whilst the apoptosis and pyroptosis inhibitors both provided only partial protection.

Necroptosis can be induced by type I IFN. Exposure of macrophages to type I IFN caused a small amount of macrophage death in a dose dependent manner (Fig.23c [2-way ANOVA, $p \leq 0.0001$]) and blocking the type I IFN response partially reduced HSV-1 dependent death (Fig.23d [2-way ANOVA, $p \leq 0.0001$]). Finally, inhibiting NF-κB signalling also increased cell survival of HSV-1 infected macrophages (Fig.23e). These data indicate that HSV-1 dependent macrophage death is partially dependent on activation of a type I IFN response and requires replicative infection.

3.3 Discussion

In this chapter I have established that HSV-1 productively infects macrophages. Macrophages exposed to HSV-1 express IE, E and L genes and proteins in a time and dose dependent fashion and nascent HSV-1 virions are produced within 48 hours of infection. HSV-1 infection of macrophages can be inhibited by soluble heparin and is sensitive to acyclovir and requires NF-κB activation. These data suggest that HSV-1 infection of macrophages mirrors HSV-1 replication in vero cells. However, macrophages were shown to be less permissive to HSV-1 than vero cells. A higher dose of HSV-1 was required to infect the same number of macrophages as vero cells, and HSV-1 infection of macrophages saturated at approximately 50-60%. This may be because monocyte derived macrophages are not a homogenous population, in the way that tissue cell lines such as vero cells are, and that within a population of macrophages some cells are unable to support HSV-1 infection, potentially because they do not express

HSV-1 entry receptors. This possibility could be assessed by staining macrophages for known HSV-1 receptors such as HVEM and nectin-1/2(405). Alternatively, as sentinel cells of the innate immune system, macrophages may resist HSV-1 replicative infection, for example by undergoing cell death or mounting a type I IFN response.

3.3.1 Cell death

I have demonstrated that HSV-1 replicative infection of macrophages induces cell death, and have described some of the cellular mechanisms involved. Cytopathic effect (CPE) has been previously observed in HSV infected primary human monocytes(230,234), U937s(238,246), THP-1s(234,247,248) and mouse peritoneal macrophages(249), but this is the first description of the mechanisms by which HSV-1 cell death occurs in human macrophages. These mechanisms are complex and given the diverse array of pro-death and anti-death signalling events that HSV-1 infection activates in other cell types, this is to be expected. HSV-1 dependent cell death in macrophages requires replicative infection, involves both the pyroptotic and necroptotic pathways and is partially dependent on NF-κB activation and engagement of the IFNR.

Caspase-1 activation and pyroptosis has been demonstrated in HSV infected melanoma cells(223). On the other hand, a number of studies have failed to observe IL-1β release following HSV-1 infection(406), the presence of which is generally considered a marker of pyroptotic death. However, it is possible for caspase-1 activation, inflammasome formation, and pyroptosis to occur in the absence of IL-1β release(138). In fact IL-1β activation and induction of macrophage cell death are orchestrated by distinct inflammasome complexes(407), the first of which contains ASC and caspase-1 that has under-gone autoproteolysis, the second lacking ASC and auto-proteolytically processed caspase-1(407). An inability to detect IL-1β as a component of the HSV-1 secretasome does therefore not rule out pyroptosis as a mechanism of HSV-1 dependent cell death. Pyroptosis can be activated by AIM2, a dsDNA sensor known to detect HSV-1(223), and this may be how HSV-1 activates caspase-1 in macrophages. To confirm this, RNAi could be used to knock-down AIM2 expression in macrophages prior to HSV-1 infection.

It has been previously suggested that HSV dependent cell death in monocytes involves necroptosis(224). U937 cells infected with a ICP4 and U$_S$3 mutant strain of HSV-1 under went cytolysis susceptible to necroptotic inhibitors(224). This mirrors HSV-1 dependent macrophage death. Necroptosis can be activated by an array of stimuli, including dsDNA, cytoplasmic RNA and type I IFN(408–412), any of which could be the PAMPs that induce necroptosis during HSV-1 infection. It would be interesting to determine whether isolated HSV-1 DNA and/or RNA can activate necroptosis in macrophages.

The type I IFN response to HSV-1 infection has also previously been linked with HSV-1 dependent cell death. U937s infected with a mutant strain of HSV lacking the gene U$_S$3 undergo apoptosis that is correlated with IRF3 activation(413). However, no study has previously shown that HSV-1 cell death in macrophages can be reduced by blocking the IFNR.

The mechanisms of HSV-1 dependent macrophage death could be further elucidated by high-throughput immunoflourescent staining. This allows single-cell analysis to confirm that cells expressing HSV-1 genes are the ones that undergo cell death. The formation of the inflammasome and the necrosome within

HSV-1 macrophages could also be confirmed by using immuno-florescence to determine the localisation of the components of these intracellular structures. It could also be investigated whether any HSV-1 factors associate with the inflammasome or necrosome. In addition, mutant HSV-1 viruses could be used to assess whether any HSV-1 encoded factors either promote or inhibit macrophage death. LAT, for example is known to protect neurons from apoptosis, whereas ICP0 promotes cell death.

It is unclear whether HSV-1 dependent macrophage death would be beneficial or detrimental to the host *in vivo*. Cell death can favour the host by eliminating viral reservoirs and activating the immune system(119–123), or favour the virus by facilitating virion release from host cells(130). Indeed, a number of HSV-1 proteins promote cell survival early during infection but activate apoptosis late on during infection, around the time of nascent virion production(414–416). Necroptosis in particular is considered a 'back-up' option for cell death that occurs only when apoptosis is suppressed, suggesting that either HSV-1 is able to inhibit apoptosis in macrophages or that macrophages are resistant to apoptotic stimuli provided by the virus. In light of this, it would be interesting to investigate whether inhibiting HSV-1 dependent cell death would inhibit or promote HSV-1 virion release.

3.3.2 Virion production

I have demonstrated that HSV-1 infection of macrophages is productive. This has been observed by some groups previously(197), although not by others, even in the presence of viral gene expression(235). Differences in the cell models may account for these contradictory data. HSV-1 virion production does appear to be sensitive to cell type, for example many groups have observed that HSV-1 can not productively infect monocytes but can productively infect differentiated macrophages or models of differentiated macrophages such as U937 cells treated with PMA(238–241), whereas the macrophages of neonates, isolated from either cord blood or the placenta, are non-permissive to HSV infection(394). Additionally, mouse peritoneal macrophages (M-Res) can not be productively infected with HSV-1(417–422). HSV-1 productive infection is even sensitive to the method of differentiation and, for example, cannot be supported in U937s differentiated with all-trans-retinoic acid, DMSO or lymphokine(239). Many of the previous investigations of HSV-1 infection of macrophages were performed in the 1980-90s, before the routine use of M-CSF and/or GM-CSF to differentiate human monocytes into macrophages. This may account for the conflicting observations between those reports and the present study. However, the numerous observations of abortive HSV-1 infection in myeloid cells suggest that HSV-1 infection of M-CSF- infected macrophages may be complex - also indicated by my observation that only 60% of monocyte derived macrophages can be infected. It may be that a proportion of macrophages are able to support abortive or quiescent HSV-1 infection and I investigate this possibility in the next chapter.

Whether or not HSV-1 infection of macrophages is productive has implications for the relevance of macrophages to HSV-1 disease. Virion release is necessary for virus spread from the mucosal epithelium to the sensory neurons and for virus transmission. If macrophages are able to support HSV-1 productive infection they may thereby contribute to both virus dissemination and transmission. If HSV-1 can also abortively infect macrophages without inducing cell death, this may also have important implications *in vivo*, for example these cells may act as reservoirs of HSV-1 infection that can be reactivated by external stimuli. Indeed, abortive infection in human monocytes can be re-activated by differentiation of the cell into a macrophage(230).

3.3.3 Type I IFN

Previous studies observed that HSV-1 infection of macrophages induces only a small amount of IFN-β release, and that HSV-1 virion production and IE expression is sensitive to treatment with exogenous IFN-β(197). My data suggest that HSV-1 in fact induces a dramatic type I IFN response in macrophages, that includes up-regulation of a range of ISGs, and that HSV-1 replication proceeds regardless. It has become increasingly evident that IFN-β release is a relatively transient event compared to ISG up-regulation(9), and this could account for the discrepancy between these data.

Recently, another study demonstrated the up-regulation of the ISGs IFNβ, IFNλ-1, TNFα, IP-10, CCL2 and CCL3 in HSV-1 infected macrophages(191). The production of IFNβ and IFNλ-1 in response to HSV-1 infection was dependent upon Mda5, but not on RIG-I or Pol III. The production of TNFα, IP10, CCL2 and CCL3 was Mda5 independent. There are therefore at least two, probably multiple(423), PRRs expressed by macrophages that are capable of detecting HSV and inducing a type I IFN and/or inflammatory cytokine response. It is also likely that HSV encodes factors that can counteract the effectors of this response, given that HSV-1 productive infection is able to occur despite its presence. It is also of interest whether or not the newly identified DNA sensor cGAS is able to detect HSV-1 infection of macrophages.

The ability of macrophages to induce both ISG up-regulation and cell death in response to HSV-1 infection may have important implications for co-infecting pathogens. HIV-1, for example, is able to infect macrophages without activating either cell death or type I IFN signalling, whilst HIV-1 replication in macrophages is sensitive to treatment with exogenous IFN-β. Given that HSV-1 and HIV-1 can both infect macrophages, it is of interest whether or not the HSV-1 dependent type I IFN response will inhibit HIV-1 replication and whether HIV-1 pre-infection will inhibit the HSV-1 dependent type I IFN response. I will address these questions in the following chapter.

3.3.4 Conclusion

I have confirmed previous observations that HSV-1 can infect macrophages. The obvious questions that arise are whether or not macrophages present at HSV-1 lesions are infected and what role they play *in vivo* infection. Mouse models of human disease have shown that macrophages are important for the control of HSV-1 encephalitis, ocular infection and hepatitis(11–13,236). It is unclear how relevant these data are to human infection, especially given that mouse macrophages are not even susceptible to HSV-1 infection(417–422). However, given the importance of macrophages to innate immunity, the well-documented presence of macrophages at HSV-1 lesions and the ability of HSV-1 to infect macrophages, it is probable that macrophages play an influential role in HSV-1 disease. This may either be as controllers of infection or as virus reservoirs. The main question to be answered is whether or not this role is beneficial or harmful to the host. Further work will be needed to elucidate the role of macrophages in human disease, however, I predict that HSV-1 encoded factors orchestrate a complex interaction with these cells, using them for different purposes at different stages of the HSV-1 infection cycle.

4 HSV-1 and HIV-1 co-infection

4.1 Introduction

In this chapter, I investigate the effect of HIV-1 pre-infection on HSV-1 lytic infection, and the effect of HSV-1 super-infection on HIV-1 infection, in human macrophages. Given the clinical and epidemiological interactions of HIV-1 and HSV-1(336,337,366), and the potential clinical importance of macrophage infection *in vivo* for both these viruses(8,252), the interaction of HIV-1 and HSV-1 at the molecular level during HIV-1/HSV-1 co-infection of macrophages is of considerable interest. This is especially the case given that both viruses may encounter each other *in vivo* in the context of macrophage infection. I therefore assess whether pre-infection of macrophages with HIV-1 affects HSV-1 IE protein expression, early and late gene transcription or the HSV-1 dependent macrophage death or type I IFN responses. I also assess whether HSV-1 superinfection affects LTR transcription and HIV-1 virion release.

In the previous chapter I demonstrated that HSV-1 lytic infection of macrophages is accompanied by cell death and a type I IFN response. HIV-1 infection of macrophages *in vitro* occurs without evident cytopathology or ISG up-regulation(9), and is sensitive to treatment with recombinant type I IFN(87,260–262). Consequently, it is of interest whether a type I IFN response to HSV-1 super-infection will have an effect on HIV-1 infection of macrophages. Given that recombinant IFN-β reduces HIV-1 virion production and replication(87,260–262), it might be predicted that HSV-1 would also negatively impact HIV-1 replication.

HIV-1 transcription can be activated by a range of co-infecting pathogens, including herpesviruses, and drugs, in particular those that activate NF-κB (Table 12). HIV-1 and HSV-1 transcription employ many of the same cellular transcription factors, and a selection of these are summarised in Table 13. Indeed, HSV-1 infection can both activate and inhibit the cellular transcription factors involved in HIV-1 replication, for example in the previous chapter I demonstrated that NF-κB translocates to the nucleus during HSV-1 infection and that this is necessary for HSV-1 transcription and HSV-1 dependent cell death to occur. However, the interaction of HSV-1 with NF-κB is context and time dependent. Both ICP0 and VP16, for example, inhibit NF-κB activation following TNF-α stimulation or TLR engagement(424–428). It is therefore difficult to predict what the net effect of HSV-1 super-infection on HIV-1 infection of macrophages will be.

As shown in Chapter 2, HSV-1 dependent cell death of macrophages involves both pyroptotic and necroptotic signalling. There is controversy over whether HIV-1 directly induces cell death in macrophages(263). There is evidence for both HIV-1 dependent cytopathology and apoptosis in macrophages(264,267,268) and HIV-1 mediated protection of macrophages from apoptosis(265,266). HIV-1 infected macrophages can also induce apoptosis of bystander cells(269,270). Additionally, both HIV-1 dependent pyroptosis and necroptosis have been demonstrated in T cells but not macrophages(429,430). Therefore, it will be interesting to see whether HSV-1 dependent macrophage death is affected by HIV-1 pre-infection, in particular whether there is a shift in the cell death signalling events that occur, for example a skewing of the response to a more apoptotic than necroptotic phenotype or whether HIV-1 is able to prevent HSV-1 induced macrophage death in the context of HSV-1 super-infection. If this were the case it could provide insights into the interactions of HIV-1 factors with macrophage cell death machinery.

A number of the restriction factors that target HIV-1 replication(431) also have activity against HSV-1. These factors include tetherin(91), members of the ABOBEC protein family(94) and, as discussed in Chapter 1, SAMHD1(293,294). Both HIV-1 and HSV-1 encode proteins that can counteract these restriction factors in certain contexts. The ABOBEC family of restriction factors, for example, is counteracted by HIV-1 vif(98,432), and ABOBECs have activity against HSV-1 when expressed at high levels(94), suggesting that HSV-1 may also encode an APOBEC antagonist that is effective against physiological levels of ABOBEC protein. Tetherin is a host restriction factor that prevents virion release by tethering nascent enveloped virions to the host cell membrane. HSV-1 glycoprotein M (gM)(91) and virion host shut-off protein (vhs)(92) and HIV-1 vpu counteract tetherin(105). Therefore, during HSV-1/HIV-1 co-infection of the same cell, there may be crosstalk between the strategies used by these viruses to counteract the activity of host cell restriction factors. This may lead to enhanced HIV-1 replication, enhanced HSV-1 lytic replication or both.

The objectives of this chapter are as follows:

1. To establish the effect of HIV-1 pre-infection on HSV-1 IE, E or late gene expression, cell death, virion release and the type I IFN response, during HSV-1 infection of macrophages.
2. To investigate the effect of HSV-1 super-infection on HIV-1 transcription and virion production in HIV-1 infected macrophages.

4.2 Results

4.2.1 HIV-1 pre-infection does not affect HSV-1 infection

To investigate the response of macrophages to HSV-1 and HIV-1 co-infection, macrophages were first infected with HIV-1 before super-infection with HSV-1. In detail, macrophages were infected with single-round HIV-1 (R9 BaL Δenv) in the presence of vpx. The infection was left to reach steady state for 6 days. The macrophages were then super-infected with HSV-1. 24 hours after infection with HSV-1, markers of HSV-1 infection were assessed, including IE protein expression, late gene transcription and cell death (Fig.24). HIV-1 infection was confirmed, and the efficiency of this infection was increased by vpx, as has previously been shown (Fig.25a [2-way [ANOVA, p=0.0036])(288). HIV-1 pre-infection had no significant effect on HSV-1 IE protein expression (Fig.25b; 2-way ANOVA, p=0.917), early gene expression (Fig.25c [2-way ANOVA, p=0.3145]), late gene expression (Fig.25d [2-way ANOVA, p=0.4975]) or production of nascent virions (Fig.26a [2-way ANOVA, p=0.5119]).

There was a significant but not systematic effect of HIV-1 pre-infection on HSV-1 dependent cell death, as assessed by the alamarBlue assay of cell metabolic activity (Fig.26b [2-way ANOVA, p=0.0020]). However, the importance of this observation is unclear given that HSV-1 dependent cell death was unaffected as determined by cell lysis (Fig.26c [2-way ANOVA, p=0.1691]) or nuclear count (Fig.26d [2-way ANOVA, p=0.9506]).

In Chapter 2, I showed that HSV-1 infection increases the nuclear area of infected cells in a way characteristic of non-apoptotic cell death, such as pyroptosis, necrosis or necroptosis. HIV-1 infection at a high MOI, 100 LTR copies per cell (LTR/cell), protected macrophages from this morphological change. The results from a single donor are showed in Fig.27. As HSV-1 dose increases the size of the nuclei increases. This effect is reduced as the dose of pre-infecting HIV-1 increases. Collating the results from 3 donors, in HIV-1/HSV-1 co-infected cells a greater proportion of cells had a normal nuclear morphology than HSV-1 infected macrophages and this effect was HIV-1 dose dependent (Fig.28b [2-way ANOVA, $p \leq 0.001$]), whereas a lower proportion of cells had the large area, low density nuclei characteristic of HSV-1 infection, and again this was HIV-1 dose dependent (Fig.28a [2-way ANOVA, $p \leq 0.0001$]). These data suggest that HIV-1 infection may interfere with an aspect of cell death signalling activated in response to HSV-1 infection. However, this effect is not sufficient to protect the macrophages from death.

Next I tested the effect of pre-existing HIV-1 infection on the type I IFN response to HSV-1 infection. In the previous chapter I showed that HSV-1 up-regulates a number of ISGs in a dose dependent manner. This response was maintained in the context of HIV-1 pre-infection (Fig.29a [2-way ANOVA, $p=0.0036$]). HIV-1 dose had no systemic effect on IP10 upregulation in the presence of HSV-1 superinfection (Fig.29a; 2-way ANOVA, $p=0.8592$). There was also no significant difference between up-regulation of RIG-I (1-way ANOVA, $p>0.05$), Mda5 (1-way ANOVA, $p>0.05$) and IFN-β (1-way ANOVA, $p>0.05$) (Fig.29b) gene expression by HSV-1 infection of macrophages compared to HSV-1 infection of macrophages in the context of HIV-1 pre-infection.

4.2.2 HSV-1 superinfection increases HIV-1 transcription

To determine if HSV-1 super-infection of HIV-1 infected macrophages affects HIV-1 LTR transcript levels over time, LTR expression levels in macrophages were determined both before and after HSV-1 super-infection. HSV-1 super-infection significantly increased LTR transcription of single-round HIV-1 in a dose dependent manner (Fig.30a [2-way ANOVA, $p \leq 0.0001$]). UV-HSV did not affect LTR transcription (Fig.30b; 2-way ANOVA, $p=0.6293$). The HSV-1 dose-dependent increase in LTR transcription was also observed in macrophages infected with full-length HIV-1 (Fig.30c [2-way ANOVA, $p \leq 0.0001$]). This was despite a robust dose-dependent type I IFN response to the HSV-1 infection (Fig.29a; 2-way ANOVA, $p=0.0036$). In fact, recombinant type I IFN also had no significant effect on HIV-1 transcription in the context of a single round HIV-1 infection (Fig.30a), although these data were variable and difficult to interpret. However, as has been previously demonstrated(9,87,260–262), IFN-β stimulation of macrophages infected with full-length HIV-1 recombinant IFN-β decreased LTR transcription compared to HIV-1 infection of macrophages in the absence of HSV-1 or IFNβ stimulation (Fig.30c [2-way ANOVA, $p \leq 0.0001$]).

4.2.3 HSV-1 prevents type I IFN dependent restriction of HIV-1 virion release

Next I investigated whether the increase in LTR transcription observed in HIV-1 infected macrophages following HSV-1 superinfection, leads to an increase in HIV-1 virion release. A p24 ELISA was performed on supernatents harvested from HIV-1 and HSV-1 co-infected macrophages, 24 hours after HSV-1 infection. HSV-1 super-infection did not significantly increase HIV-1 release from HIV-1 infected macrophages. However, it has previously been demonstrated that type I IFN decreases HIV-1 virion release. We confirmed this observation, IFN-β decreases HIV-1 virion release by macrophages compared to HIV-1 virion release by macrophages not stimulated with IFN-β or super-infected with

HSV-1 (Fig.30d [2-way ANOVA, $p \leq 0.0001$]). Given that super-infection with HSV-1 of HIV-1 infected macrophages induces a type I IFN response, it may have been expected that this interferon response would inhibit HIV-1 release. However, this was not the case. On the contrary HSV-1 super-infection increased virion release in a dose-dependent manner (Fig.30d [2-way ANOVA, $p=0.0062$]). Therefore, it appears that HSV-1 super-infection protects HIV-1 virion release by HIV-1 infected macrophages from the HSV-1 dependent type I IFN response.

4.3 Discussion

In this chapter I have shown that pre-infection with HIV-1 does not affect HSV-1 replicative infection. HSV-1 IE, E and late gene expression is unaffected, as is virion production, HSV-1 dependent macrophage death and HSV-1 dependent up-regulation of ISGs. HSV-1 induces nuclei morphological changes of infected cells characteristic of non-apoptotic cell death. HIV-1 pre-infection appears to partly protect macrophages from this effect. However, it is unclear how relevant this is, given that HSV-1 dependent macrophage death appears unaffected. I have also shown that HSV-1 super-infection increases HIV-1 LTR transcription in a dose dependent fashion, in macrophages infected with either single-round or full-length HIV-1. This effect is dependent on HSV-1 replication and occurs within 24 hours of HSV-1 infection. By this time HSV-1 IE, E and late gene expression has occurred.

4.3.1 HSV-1 mediated enhancement of HIV-1 transcription

HIV-1 transcription from the integrated provirus involves both viral and cellular factors, as summarised in Tables 7 and 8. HIV-1 transcription occurs by recruitment of the cellular RNA polymerase II (RNAPII) to the LTR promoter. RNAPII initiates basal transcription from the LTR but then pauses after synthesis of a short transcript that includes TAR(433). Tat binds a stem loop structure within TAR(434) and recruits P-TEF-b. CDK9, a component of P-TEF-b(435), phosphorylates the RNAPII carboxyterminal domain (CTD) and enables RNAP mediated productive elongation of the HIV-1 transcript(436). An HIV-1 enhancer element in the LTR promoter can be bound by a number of cellular transcription factors that enhance HIV-1 transcription, including NF-κB, Sp1 and NF-AT.

Previous reports have demonstrated increased HIV-1 transcription following infection with a range of co-infecting pathogens, including herpesviruses. The KSHV IE protein ORF50(437), for example, interacts synergistically with tat to enhance LTR transcription(438,439). NF-κB is a potent transcriptional activator of the HIV enhancer(440) and the VZV IE4 protein trans-activates LTR transcription via a NF-κB dependent mechanism(441), whereas the EBV EBNA2 protein, important for the establishment and maintenance of EBV latency in B cells(442), enhances LTR transcription via a Sp1 and NF-κB dependent mechanism(443). NF-κB activation is also the mechanism by which HHV-6(444,445), HBV HBX, *Mycobacterium tuberculosis* Rv1168C protein(446–448), *Neisseria gonorrhoeae*(449–451), *Cryptococcus neoformans*(452), *Toxoplasma gondii*(453) and methamphetamines(454) increase HIV-1 LTR transcription. Given that HSV-1 activates NF-κB during replicative infection, as demonstrated in Chapter 2, this may also be the mechanism by which HSV-1 enhances HIV-1 transcription. Unfortunately this hypothesis is difficult to test, given that NF-κB signalling is necessary for the progression of HSV-1 infection, and that HSV-1 replicative infection is required for the enhancement of LTR transcription. To take this work further it will therefore be necessary to identify the HSV-1 factor or factors responsible for the effect.

Many HSV-1 encoded proteins interact with the host transcriptional machinery, providing multiple candidates for the one or more involved in enhancing LTR transcription. Mutant HSV-1 strains, lacking specific functional HSV-1 proteins, could be screened for the lack of this ability. However, here again there is the difficulty of distinguishing a direct effect from the indirect effect resulting from reduced HSV-1 replication. Despite its role in facilitating HSV-1 transcription, ICP0 can be ruled out as a potential candidate, given that it appears to inhibit rather than activate NF-κB, for example in response to TNF-α stimulation(424,425). HSV-1 glycoproteins are more promising candidates. gD, for example, inhibits HSV-1 dependent apoptosis in U937 by activating NF-κB(455). U$_L$31 has also been shown to be necessary for optimal NF-κB activation(456), and is another potential candidate.

4.3.2 HIV-1 virion release and type I IFN

Increased HIV-1 transcription in response to a co-infecting pathogen or drug treatment is a commonly observed phenomenon. Pathogen or drug mediated enhancement of HIV-1 virion release is seen less frequently. It has been demonstrated for example in response to noradrenaline treatment(457). I did not observe an increase in HIV-1 virion release to mirror the HSV-1 dependent enhancement of LTR transcription, although a small significant dose-dependent effect was observed.

In line with previous studies, I observe that exposure of HIV-1 infected macrophages to type I IFN inhibits both HIV-1 transcription and virion release(8), due to the antiviral effects of multiple ISGs such as the retrovirus restriction factors MX2(458),TRIM22(459) and tetherin(105). However, I did not observe a decrease in either HIV-1 transcription or virion release in response to an HSV-1 dependent type I IFN response, despite this response involving up-regulation of multiple ISGs, including Mda5, IFI16, IFN-β and IP10, and engagement of the IFNR. In fact, as described above, HIV-1 transcription increases in response to HSV-1 infection. Therefore, it may be hypothesized that HSV-1 is able to promote both HIV-1 transcription and virion release, either by independently activating LTR transcription and protecting HIV-1 virion release type I IFN mediated inhibition, or by enhancing HIV-1 replication such that the effect of type I IFN on HIV-1 transcription and virion release is masked. To investigate these hypotheses, the type I IFN response to HSV-1 could be inhibited during co-infection, to determine if HIV-1 virion release is enhanced by co-infection in this context.

The ability of HSV-1 to increase HIV-1 transcription during HIV-1/HSV-1 macrophage co-infection may provide a mechanism for some of the clinical synergy between these two viruses. HSV infection is correlated with increased HIV-1 transmission and disease severity(14,15,104,324–338). HSV-1 superinfection of HIV-1 infected macrophages at the HSV-1 transmission site, for example the vaginal mucosa, may enhance HIV-1 replication by protecting it from the type I IFN response mounted by the host, and thereby enhancing HIV-1 replication within the host and increasing the risk of HIV-1 transmission.

4.3.3 HIV-1 dependent protection from changes in nuclear morphology

As demonstrated in Chapter 2, the cell death response to HSV-1 infection is complex. It occurs by a non-apoptotic mechanism, as indicated by the large dim nuclei of the dying cells that contrast with the small intense nuclei of apoptotic cells(460). HSV-1 macrophage death involves both pyroptotic and necroptotic signalling. Pre-infection with HIV-1 does not protect macrophages from HSV-1 dependent cell death. However, it does appear to partly protect the nuclei of these cells from morphological change. This is perhaps not surprising, given that HIV-1 has been shown to have a complex relationship with the cell death machinery and interacts with apoptotic, necroptotic and pyroptotic signalling (265,266,429,430). However, it is unclear whether the effect of HIV-1 on HSV-1 induced nuclear morphology has any functional relevance, for example whether the effect observed is the result of slightly delayed cell death or represents a skewing of the cell death response from one pathway to another, for example from necroptotic to apoptotic. To investigate this further, specific inhibitors of cell death signalling could be used to see if the phenotype of HSV-1 dependent cell death reported in Chapter 2, is different in the context of HIV-1 co-infection.

4.3.4 Conclusion

I have now established that HSV-1 can productively infect macrophages and that pre-infection with HIV-1 has little effect on this infection. Also, that HIV-1 super-infection of HSV-1 infected macrophages increases HIV-1 transcription. In the next chapter, I will investigate whether HSV-1 can establish a latent infection of macrophages and whether HIV-1 super-infection has any effect on this, as well as whether the HSV-1 pre-infection has any effect on the HIV-1 infection itself.

5 HSV-1 latency in macrophages

5.1 Introduction

In this chapter I investigated if and how HSV-1 establishes latency in macrophages, where latency is defined as abortive infection that can be reactivated.

HSV-1 has both a lytic and latent replication cycle(151). The molecular mechanisms by which HSV-1 latent infection of neurons is thought to occur are described in Chapter 1. The ability to establish static latent infection has significant advantages for a virus. Latency enables virus persistence within a small host population(461). Limited or lack of viral replication reduces the cost of infection in the context of a mutualistic interaction with the host and host survival increases the opportunities for viral transmission(462). There is little doubt that the herpesviruses are some of the most successful of pathogens, primarily due to their ability to persist, with occasional reactivation, throughout the entire life of the host(461). Latency is also the reason that, although we have effective antivirals against HSV-1, the infection remains incurable, and that there is currently no vaccine(463). Current dogma is that α-herpesviruses such as HSV-1 and VZV only establish latency within neurons(159). However, abortive HSV infection can be established in other cell types *in vitro* (Table 14)(464,465). Given the presence of macrophages at HSV-1 lesions(250,251) and their importance for the control of HSV-1 disease(11–13,236,237,252), HSV-1 abortive infection and reactivation within macrophages would have profound implications for the treatment and eradication of HSV-1.

In Chapter 2, I describe HSV-1 lytic infection of macrophages. This infection proceeds as it does in epithelial cells, with a sequential pattern of gene expression followed by virion release and cell death(151). However, there are differences between HSV-1 lytic infection of vero cells and HSV-1 infection of macrophages, which in combination suggest that a proportion of a macrophage population exposed to HSV-1 may support HSV-1 abortive or latent infection. First, a higher dose of HSV-1 is required to infect macrophages than vero cells, suggesting that a proportion of macrophages are either resistant to HSV-1 infection entirely, for example because they lack the necessary entry receptor(s), or can only support abortive infection. Second, when exposed to HSV-1, a greater percentage of macrophages express the IE proteins ICP0 and ICP4 than undergo HSV-1 replication dependent cell death. Again this is suggestive of an abortive infection. IE protein expression has been observed in neurons latently infected with HSV-1(466) and ICP0 may play a role in maintenance of HSV-1 latency(390–392,467,468). Finally, the intracellular localisation of ICP0 in the cytoplasm, and not the nucleus, within macrophages is unusual. ICP0 has previously only been observed in the cytoplasm during HSV-1 latency in neurons(469) or very late on during lytic infection(470).

Macrophages are similar in a number of ways to neurons, the canonical site of HSV-1 latent infection, in that they are terminally differentiated non-cycling cells highly resistant to apoptotic stimuli(471–474). Interestingly, both the non-cycling state and apoptotic resistance of neurons has been proposed to underlie their capacity to support HSV-1 latency(221,475–477).

LAT is the only gene expressed during HSV-1 latent infection of neurons(478–480). Although it is not essential for the establishment of latency(481), it does play a role in latency maintenance and reactivation(482,483). LAT is also expressed in HSV-1 infected macrophages. There are two forms of LAT that can be stably expressed, the 1.5 kilobases (kb) stable intron and the 2.0kb stable intron(484) (Fig.31). The 2.0kb intron is expressed at high levels during both lytic and latent infection, whereas the 1.5kb LAT is expressed only during latency(484). These transcriptional states are distinguishable by Northern blot analysis(479,485) but not by RT-qPCR, as the 1.5kb LAT intron is entirely encoded within the 2.0kb intron(484). Consequently, detection of LAT in HSV-1 infected macrophages supports, but does not prove, that HSV-1 can establish latency in these cells.

Latency is often defined as the presence of viral genomes in the absence of lytic gene expression or virion production. Therefore, I assess lytic gene expression, virion production and LAT expression over time in HSV-1 infected macrophage cultures. If latency is established, LAT expression would persist whilst lytic gene expression diminished due to HSV-1 dependent macrophage death.

It could be argued that true latency is defined as abortive infection that can be reactivated. This is certainly the definition used for current *in vitro* models of latency (Table 14), for example, one well established model is to infect neurons in the presence of ACV, such that replicative infection is stalled at viral DNA replication, and to reactivate replicative infection by withdrawing the drug(256,486,487). I therefore attempt to reactivate abortive HSV-1 infection in macrophages. An interesting stimulus to test in this context is HIV-1 infection, given the nature of the complex interaction between macrophages and HIV-1 and the degree of clinical and epidemiological interaction between HSV-1 and HIV-1(343). These interactions are described in detail in Chapter 1. Furthermore, if macrophages are latently infected with HSV-1 *in vivo* at mucosal tissue sites, HSV-1 may encounter HIV-1 during HSV-1/HIV-1 coinfection.

The objectives of this chapter are as follows:

1. To establish if macrophages can be latently infected with HSV-1 by assessing cell survival, virion production, LAT expression and late gene expression in HSV-1 infected macrophages over time.
2. To begin to investigate the mechanisms by which HSV-1 latency in macrophages is established, paying particular attention to the role of type I IFN.
3. To determine if HSV-1 quiescent infection of macrophages can be reactivated with HIV-1, and to begin to investigate the mechanisms by which this may occur.

5.2 Results

5.2.1 HSV-1 latently infects macrophages

If, within a macrophage population exposed to HSV-1, a proportion of macrophages supports HSV-1 latency whilst others support HSV-1 lytic replication, we would expect gB expression within the population to diminish over time, as productively infected cells die. LAT expressing cells would persist (Fig.32). Macrophages infected with a medium to low dose of HSV-1 survived until at least 2 weeks post infection. There was no difference over a period of 2 weeks between the nuclear count (Fig.33a [2-way ANOVA, $p=0.1167$]) or the level of cell metabolism (Fig.33b [2-way ANOVA, $p=0.6089$]) of macrophages infected with either 2 or 20pfu$_{vero}$/cell of HSV-1. Also, there was no difference in detectable cell lysis of macrophages 2 weeks after infection with either 2 or 20pfu$_{vero}$/cell of HSV-1, compared to cell lysis 2 days after infection (Fig.33c [1-way ANOVA, $p>0.05$]).

As previously observed, after 24 hours infection with HSV-1, both gB and LAT expression were high (Fig.34a and Fig.34b). However, over a 2 week period gB expression decreased in macrophages infected with HSV-1 in a time dependent manner (Fig.34a [2-way ANOVA, $p \leq 0.0001$]). gB expression had all but disappeared 2 weeks after infection with either 2pfu$_{vero}$/cell (Fig.34a [1-way ANOVA, $p \leq 0.0001$]) or 20pfu$_{vero}$/cell (Fig.34a [1-way ANOVA, $p \leq 0.0001$]). LAT expression, on the other hand, remained high over 2 weeks after infection with either 2pfu$_{vero}$/cell (Fig.34b [1-way ANOVA, $p > 0.05$]) or 20pfu$_{vero}$/cell (Fig.34b [1-way ANOVA, $p > 0.05$]). Expression of LAT did change significantly in a time dependent manner (Fig.34b [2-way ANOVA, $p \leq 0.0001$]), but not in a systematic fashion. It went down and then up.

GAPDH and IP10 expression levels were relatively stable over the two weeks (Fig.34c [1-way ANOVA $p > 0.05$] and Fig.34d [2-way ANOVA, $p = 0.1123$]). These data suggest that a proportion of macrophages do indeed support abortive HSV-1 infection.

5.2.2 Establishment of latency

To determine whether the establishment of HSV-1 latency in macrophages required an initial round of productive infection, LAT and gB expression over time was assessed in macrophages exposed to HSV-1 in the presence of acyclovir (Fig.35). As shown in Chapter 2, acyclovir inhibits HSV-1 DNA replication and lytic gene expression in macrophages. Acyclovir abolished gB (Fig.36a [2-way ANOVA, $p \leq 0.0001$]) expression, suggesting that HSV-1 replication is indeed required for the establishment of latency.

In Chapter 2, I demonstrated that HSV-1 replicative infection activates a type I IFN response. To assess whether the type I IFN response to HSV-1 lytic infection contributed to the establishment of latency during secondary HSV-1 infection, I used an IFNR blocking antibody during the initial HSV-1 infection (Fig.37). Blocking the IFNR inhibited the type I IFN response after 24 hours to both HSV-1 infection and treatment with IFN-β (Fig.38a). IP10 up-regulation was not observed over the 2 weeks following treatment with IFN-β in the presence of the antibody compared to treatment with IFN-β in the absence of the antibody (Fig.38a [2-way ANOVA, $p \leq 0.0001$]). However, IP10 up-regulation was observed within a week of HSV-1 infection in the presence of the antibody compared to IP10 up-regulation after 1 day post infection with HSV-1 in the presence of the antibody (Fig.38a [1-way ANOVA, $0.05 \leq p > 0.001$]). This was probably due to a HSV-1 dependent type I IFN response to continued HSV-1 lytic replication following withdrawal of the antibody after 24 hours. There was little or no significant difference between either gB (Fig.38b [2-way ANOVA, $p = 0.5489$]) or LAT (Fig.38c [2-way ANOVA, $p = 0.3674$]) expression following HSV-1 infection in the presence or absence of the blocking antibody. Unfortunately, due to time constraints, I was unable to assess the effect of inhibiting NF-κB or blocking the IFNR during the full 2 weeks over which latency was established.

5.2.3 Latent HSV-1 is reactivated by HIV-1

By definition, a true latent infection can be re-activated to a lytic infection. In Chapter 3, I demonstrated that HIV-1 pre-infection has no effect on replicative HSV-1 infection, and that HSV-1 superinfection increases HIV-1 transcription. Given the role of NF-κB signalling in both HIV-1 and HSV-1 transcription, I attempted to re-activate latent HSV-1 from macrophages either by HIV-1 infection or LPS stimulation. LPS is an outer membrane component of Gram negative bacteria that activates NF-κB signalling via

TLR4 stimulation[488]. LPS can reactivate other herpesviruses from latency[489] and is a very potent stimulator of macrophages. Given the importance of NF-κB signalling in HSV-1 transcription, it may be expected that LPS would reactivate latent HSV-1 infection of macrophages. LAT$^+$ gB$^-$ macrophages were stimulated either with LPS, vpx only or single-round HIV-1 with vpx, 2 weeks post infection with HSV-1 (Fig.39). As expected, after an initial burst, gB expression decreased over time until it had all but disappeared by 2 weeks (Fig.40a). Stimulation with LPS at this point had no effect on gB expression (Fig.40a [1-way ANOVA, $p>0.05$]), but up-regulated expression of IL-6 as has been previously shown (Fig41a [2-way ANOVA, $p\leq0.0001$]). However, stimulating HSV-1 infected macrophages with HIV-1 caused an increase in gB expression over a 24 hour window (Fig.40a [2-way ANOVA, $p=0.0036$]), suggesting that HIV-1 can re-activate latent HSV-1 in macrophages. These data were mirrored by those for HSV-1 DNA Pol expression over time and for the effect of HIV-1 infection on DNA Pol expression at 2 weeks post infection with HSV-1 (Fig.40b [2-way ANOVA, $p=0.0141$]).

The supernatents of macrophages latently infected with HSV-1 were tested for the presence of viable viruses, both before and after HIV-1 stimulation. Prior to HIV-1 stimulation, hardly any viable virus was detectable in the supernatent of latently infected macrophages. After HIV-1 stimulation, viable HSV-1 virions were released within 24hpi (Fig.40c [1-way ANOVA, $0.01<p\leq0.05$]). IP10 transcription was unaffected by time (Fig.40d [2-way ANOVA, $p=0.00123$]) or HSV-1 re-activation (Fig.40d [1 way ANOVA, $p>0.05$]), remaining high throughout.

5.2.4 HIV-1 transcription is not required for reactivation of HSV-1 latency

Exposing macrophages latently infected with HSV-1 to HIV-1 prevented HIV-1 LTR transcription (Fig.41b [1-way ANOVA, $p\leq0.0001$]). This was not surprising, given that HIV-1 infection is sensitive to type I IFN[9] and that latently infected macrophages still displayed significant ISG up-regulation 2 weeks post infection with HSV-1 (Fig.34d). Furthermore, exposing macrophages latently infected with HSV-1 or mock infected macrophages to UV inactivated HIV-1 (UV-HIV-1) such that no LTR transcription was observed in the mock infected macrophages (Fig.41c), resulted in little or no reactivation from macrophages infected with either 2pfu$_{vero}$/cell (Fig.41d [1-way ANOVA, $p>0.05$]) or 20pfu$_{vero}$/cell (1-way ANOVA, $p>0.05$). Although there was a small significant systematic effect (2-way ANOVA, $p=0.444$).

5.3 Discussion

In this chapter I have shown that HSV-1 can establish latency in macrophages, where latency is defined as a quiescent or abortive infection that can be reactivated. A proportion of cells within a population of macrophages exposed to HSV-1 support replicative infection incorporating late gene expression, virion production, a type IFN response and cell death. Another proportion of macrophages support quiescent or abortive infection, comprising LAT expression, no late gene expression, no virion production and a type I IFN response. These gB$^-$LAT$^+$ macrophages survive for at least 2 weeks. HSV-1 latency in macrophages is dependent on the initial round of replicative infection, as acyclovir treatment during the first 24 hours of infection abolishes not only gB expression and cell death, but also LAT expression. Finally, exposure of gB$^-$LAT$^+$ macrophages to HIV-1, and to a lesser degree UV-HIV-1, re-activates gB expression and virion production, in the absence of HIV-1 transcription.

5.3.1 Establishment of latency

Latent HSV-1 infection has previously been modelled in neuron cell lines and primary neurons, where an abortive infection established in the presence of an inhibitor is reactivated by removing the inhibitor. One such model involves infecting PC12 rat cells with HSV-1 in the presence of nerve growth factor (NGF)(490). In this experimental system, HSV-1 DNA and LAT is detectable and there is little or no virion production. Removal of NGF causes an increase in virion production(490). It is thought that the ability of NGF to promote the establishment of latency is linked to its anti-apoptotic effect, in line with the ability of promotors of apoptosis to reactivate latent HSV-1(221,491). Another model of HSV-1 latency is the infection of PC12 cells with HSV-1 in the presence of acyclovir(487,492). This promotes the establishment of an infection with no detectable virus production. Latency is maintained when acyclovir is withdrawn after 10 days(487,492). In this model, latency can be reactivated by heat stress(487) or forskolin treatment(492). Forskolin activates adenylyl cyclase and increases the intracellular level of cAMP(493). Another method of modelling HSV-1 latency *in vitro* is to infect human fetus dorsal root ganglion (DRG) neurons with HSV-1 in the presence of an anti-HSV drug, for example the thymine analogue (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVdUrd) a , and IFN-α(477). These *in vitro* models of HSV-1 latency mirror HSV-1 latency *in vivo* in that they can be reactivated by stress, that they involve the expression of LAT in the absence of lytic gene expression or virion production, that LAT is not essential for the establishment of latency and that they are sensitive to deletion of the same HSV-1 proteins, for example U$_S$5, and that these deletions can be compensated for with the expression of LAT(494). HSV-1 quiescent infection of macrophages is similar to these previous *in vitro* models, in that virion production is absent, LAT is expressed and productive infection can be reactivated. As such, it is reasonable to term HSV-1 quiescent infection of macrophages as latency, according to how the term is currently used in the field.

HSV-1 latent infection of macrophages as a model of HSV-1 latent infection possesses a number of advantages over previous *in vitro* models. Firstly, establishing a quiescent HSV-1 infection of macrophages does not require anti-HSV-1 drugs, recombinant type I IFN or anti-apoptotic growth factors. Instead, HSV-1 infection of macrophages appears to mirror that which occurs during HSV-1 infection of neurons *in vivo*, in that there is an initial round of lytic replication, induction of innate immune responses, virion production and cell death. As in neurons, this infection is gradually dampened, such that productively infected macrophages die and gB$^-$LAT$^+$ cells persist. Consequently, HSV-1 infection of macrophages provides a model of HSV-1 infection that includes both the lytic and latent replicative cycles, and, with the lack of a need for anti-HSV-1 drugs, is arguably a more relevant model of HSV-1 latency than previous experimental models.

Given the ability of type I IFN to induce HSV-1 latency in other experimental models(491), the type I IFN response to HSV-1 infection of macrophages and the necessity for an initial round of replicative infection required for the establishment of HSV-1 latency in macrophages, I propose a model of how HSV-1 latency may be established in this system. Infecting macrophages with a high dose of HSV-1 causes a close to 100% lytic infection, resulting in virion production and cell death within 48hpi. Infecting macrophages with a low to medium dose of HSV-1 results in a less than 100% infection (Fig.42a). Here, the infected cells undergo lytic replication. Within 6 hours they also release type I IFN that has a paracrine effect on the bystanding uninfected cells (Fig.42b). Nascent virions are released after 24 hours. These infect the bystander cells in which an antiviral state has already been established (Fig.42c). I propose that HSV-1 is unable to undergo replicative infection under these conditions and instead establishes a latent infection (Fig.42d).

I have attempted to determine if either NF-κB signalling or IFNR engagement is necessary for the establishment of HSV-1 latency in macrophages. However, the initial replicative infection is sensitive to NF-κB inhibition, as demonstrated in Chapter 2, and the establishment of latency is dose dependent on the degree of replicative infection that occurs in the macrophage culture. Therefore, it is unclear whether inhibiting NF-κB effects the establishment of latency directly or indirectly by reducing the initial round of replicative infection. This aspect of the infection also makes it difficult to assess the effect of recombinant type I IFN on the establishment of latency, as replicative HSV-1 infection is also sensitive to type I IFN. Additionally, replicative infection and the type I IFN response persists over the 2 week period that latency is established, making it difficult to block it completely by a single intervention. This again makes it difficult to interpret the results of these experiments.

HSV-1 latent infection of macrophages provides considerable opportunity for investigating the mechanisms of latency maintenance. It could be investigated, for example, whether LAT is necessary and/or sufficient for the phenotype, and whether it performs similar roles in macrophages as it does in neurons. These include, protecting latently infected cells from HSV-1 superinfection(495), repressing IE transcription(483), promoting cell survival potentially by HVEM up-regulation(496) and activation of NF-κB and type I IFN signalling(497). The function of LAT during HSV-1 infection of macrophages could be assessed using a HSV-1 mutant virus lacking the ability to express LAT(498). Additionally, HSV-1 latency of macrophages could be further characterised. Of particular interest is whether the viral genomes in latently infected macrophages are circularised as in latently infected neurons, how many of the population of surviving macrophages express LAT and/or contain viral DNA and whether any HSV-1 proteins persist in the latently infected macrophages, in particular ICP0. These questions could be addressed using immuno-florescence to detect HSV-1 protein and fluorescence *in situ* hybridization (FISH) to detect HSV-1 DNA and LAT(499).

During HSV-1 infection of neurons, VP16 is restricted to the cytoplasm, and so can not activate IE gene expression, as described in Chapter 1. It is thought that this effect is due to the non-cycling state of neurons. It would be interesting to assess the localisation of these factors in macrophages, and to assess the intracellular localisation of VP16 in macrophages both lytically and latently infected with HSV-1, so as to determine if the lack of VP16 mediated ICP0 expression provides a block to HSV-1 replicative infection in macrophages.

The second block to HSV-1 replicative infection in neurons is at the ICP0 mediated expression of other IE genes. ICP0 is required to remove the HDAC-1-CoREST-REST repressor complex from the IE gene promoters. ICP0 can be inhibited from performing this function by being trafficked to the cytoplasm. Consequently, the intracellular localisation of ICP0 in macrophages latently infected with HSV-1 is also of interest.

5.3.2 Reactivation of latency

HIV-1 mediated reactivation of HSV-1 latency is a novel observation. Methods of reacting latent HSV-1 *in vitro* have included heat stress(492), cold shock(465,477,500–502), exposure to inhibitory drugs or growth factors(256,486,487,503), expression of IE proteins from adenovirus vectors(487) or UV irradiation(504). It is of interest whether any of these stimuli could also reactivate HSV-1 latency in macrophages.

Further work is required to characterize the nature of the reactivated HSV-1 infection. I have shown that it involves both late and early gene expression and virion release. It will be interesting to determine whether IE proteins are expressed, or whether this part of HSV-1 replicative infection is bypassed during reactivation in macrophages. There are precedents for this, for example within experimental models of latency where latency is reactivated by factors that preform the action of IE HSV-1 proteins. It will also be interesting to establish whether viral DNA replication is stalled during establishment of HSV-1 latency in macrophages and whether it is resumed during reactivation, and in connection with this, what the form of the HSV-1 DNA takes in the latently infected macrophages, i.e. whether it is in a circularised or linear form.

Super-infection with another virus has been shown to reactivate HSV-2 latency(464). In this experimental model, HSV-2 quiescent infection of human embryonic lung cells *in vitro* was reactivated by HCMV super-infection and E gene expression(464). Both HSV-1 and HCMV IE and E protein share the capacity for both viral and cellular gene transactivation, for example by recruiting NF-κB and other host transcription factors. HIV-1 proteins also interact with the host cell transcription machinery (Table 7). It may be that in the context of HIV-1 reactivation of HSV-1, HIV-1 factors establish a transactivational state, for the purpose of promoting HIV-1 and cellular gene expression, that has the bystander effect of promoting HSV-1 replicative gene expression. This is supported by a model of HSV-1 latency whereby a general state of viral gene silencing is established and can be reactivated to gene expression by the expression of HSV-1 IE proteins(487), pro-apoptotic drugs, stress(492) or histone deacetylase inhibitors(256).

Given that exposure to HIV-1 can reactivate latent HSV-1 from macrophages without detectable HIV-1 LTR transcription, and that UV-HIV-1 can cause partial reactivation, the ability of HIV-1 to reactivate HSV-1 must be dependent on a component or entry process of the HIV-1 virion. The process of virion entry itself is known to activate cells(505,506), for example by inducing membrane permutations that activate STING(507). Therefore, a key issue to clarify in this work is whether entry of other VSV-pseudotyped lentiviruses, virus-like particles (VLPs) or enveloped viruses, such as adenoviruses, are capable of reactivating HSV-1 latency in macrophages. However, given that vpx, which is also delivered via a VSV-pseudotyped lentivector, does not reactivate HSV-1, the effect of HIV-1 may be more specific than this. The effect of empty VSV-pseudotyped lentivectors or VLPs should also be assessed as the most appropriate control for the specificity of the HIV-1 mediated effect.

The HIV-1 p24 capsid contains two copies of the ssRNA genome tightly bound by the nucleocapsid proteins, p6, p7, and the virally encoded RT and integrase. A matrix of p17 surrounds the capsid. The HIV-1 accessory proteins vif, vpr and nef are also contained in the virion(508,509). Vif, vpr and nef are all multifunctional proteins, the known functions of which are described in Table 15. Any of these three proteins may be responsible for HIV-1 dependent reactivation of HSV-1. Vif is essential for HIV-1 infection of its target cells(510,511). Its primary function is to prevent ABOBEC mediated restriction of HIV-1 replication(96–104,432,512–514). HSV-1 infection is also sensitive to APOBEC cytidine deaminases both *in vitro*(94) and in animal models of HSV-1 encephalitis(93), suggesting that vif may be able to promote HSV-1 infection. However, it is unclear how this would affect HSV-1 latency. Vpr is a more likely candidate. Vpr is not required for virus replicationT cells but enhances the ability of the virus to infect macrophages(515,516). The functions of vpr include interacting with Sp1 to transactivate HIV-1 LTR expression(517) and reactivating HIV-1 virion production from latency(518). Sp1 is activated during HSV-1 infection in an ICP4 dependent manner(519) and it may be that vpr is able to substitute for this function of ICP4 in order to reactivate latent HSV-1. Alternatively, vpr may enable HSV-1 reactivation by inducing a global transcriptionally active state within the co-infected macrophages, in the same way that it reactivates HIV-1 virion production and increases hepatitis C virus RNA replication(518,520). Vpr can also induce apoptosis(521,522). Apoptosis has been shown to induce HSV-1 reactivation in ganglionic organ cultures(221), suggesting that this is another potential mechanism by which HIV-1 may reactivate HSV-1. Additionally, both vif and vpr manipulate the cell cycle to favour HIV-1 replication(523–527). Given the known importance of the mitotic state of HSV-1 target cells for the determination of whether replicative or latent infection is established, these functions of vif and vpr may also contribute to HIV-1 dependent reactivation of latent HSV-1 in macrophages. Nef enhances HIV-1 virion infectivity and increases HIV-1 replication in T cells and macrophages(528). In contrast to vpr, nef protects macrophages from apoptosis(529–535), a function shared by HSV-1 LAT(536–538). Indeed, another anti-apoptotic gene, the bovine herpes virus 1 (BHV-1) latency-related (LR) gene, is able to efficiently substitute for LAT function in HSV-1 reactivation(538). Finally, like vpr, nef is able to activate an array of transcription factors, including AP-1, NF-κB, STAT1 and STAT3(529,539–541). NF-κB activation can be ruled out as a potential mechanism of HSV-1 reactivation in macrophages, given that LPS, a potent activator of NF-κB, has no effect on latent HSV-1. Potentially, the combinatorial effect of vpr, vif and nef enables HIV-1 to reactivate HSV-1. I would predict that the most likely mechanism is that HIV-1 capsid proteins manipulate the infected cellular environment to produce a transcriptionally active state, that indirectly enables HSV-1 to bypass a block to replicative infection.

5.3.3 Conclusion

Quite apart from being a novel model of HSV-1 latency, HSV-1 quiescent infection of macrophages may be relevant to *in vivo* infection, where tissue macrophages may provide additional reservoirs of infection. This would have implications for efforts to eradicate HSV-1 infection from the body and for strategies aimed at preventing HSV-1 reactivation. However, it is likely that only a few macrophages, for example those present at the transmission site, will be latently infected with HSV-1 in healthy individuals. This will make it difficult to investigate HSV-1 latent infection of macrophages *in vivo*. A difficulty compounded by the nature of latency itself, definitionally the lack of viral gene expression, that has presented difficulties to herpes-virologists for decades. To further complicate issues, it is unlikely that mouse models of HSV-1 disease will be of use, given that HSV-1 is unable to infect mouse macrophages. It may be that methods that can track a virus infection, including the cell type that are subsequently infected, as it progresses through the body(542) will provide novel insights into which cells

HSV-1 can and does infect *in vivo*.

The ability of HIV-1 to reactivate latent HSV-1 infection of macrophages may have profound implications for our understanding of the interaction of these viruses within a co-infected individual. HIV-1 co-infection increases HSV-1/2 shedding, HSV-1/2 reactivation rates and the duration of hepatic ulcers(176,340,350,354–357). It has previously been thought that this is due to HIV-1 mediated disruption of the host immune system.(343,356,357). However, it may also be that HIV-1 and HSV-1 interact directly within host cells, and that HIV-1 is able to reactivate latent HSV-1 in macrophages at HIV-1/HSV-1 transmission sites. This may be a mechanism by which HIV-1 increases the severity of HSV-1 associated disease(366). The majority of the epidemiological studies of HSV/HIV-1 co-infection concentrate on HSV-2. HSV-1 is now the leading cause of genital herpes in the developed world, and it is reasonable to believe that HSV-1 will or does have the same epidemiological interactions with HIV-1 as HSV-2. However, the current study could be extended to incorporate to HSV-2 and to investigate whether it too can establish a latent infection in macrophages.

6 General discussion

HSV-1 and HIV-1 co-infection of macrophages is an interesting avenue of research in regard to dissecting the mechanisms by which the innate immune system responds to viral infection *in vivo*. It is also relevant to the understanding to host pathogen interactions between these clinically significant viruses and their target cells. Additionally, the considerable clinical interaction between HSV-1 and HIV-1 at the population and epidemiological levels, prompts a closer examination of the potential interactions of these viruses at the cellular and molecular level.

In this thesis, I confirm previous observations that HSV-1 productively infects human macrophages. Previous reports have failed to extensively characterise this infection and have generally been restricted to the measurement of only a few aspects of HSV-1 replication, in particular IE protein expression, nascent virion production or cell death. However, it is becoming increasingly evident from investigations of HSV-1 latency and abortive infection, that the HSV-1 replication cycle can stall following IE expression and that the absence of cell death does not necessarily indicate a lack of productive infection. In contrast to these previous studies, I demonstrate HSV-1 productive infection of macrophages at the levels of IE, E and late gene expression, IE and late protein expression, nascent virion production and cell death. This provides very clear evidence that macrophages are permissive to HSV-1 replicative infection. It follows that tissue macrophages, at the site of HSV-1 lesions, are likley targets for the virus *in vivo*. Unfortunately, this hypothesis is difficult to test, particularly because mouse macrophages are resistant to HSV-1 infection, primary human macrophages are difficult to obtain and tissue sections of HSV-1 lesions are not readily available for histochemical staining.

HSV-1 nucleic acids can be detected *in vitro* by the majority of the cytoplasmic nucleic acid sensors so far discovered. However, this was demonstrated in studies using models in which a particular receptor of interest was over-expressed in a tissue cell line. It could be argued that these systems may not tell us much about cytoplasmic nucleic acid sensing *in vivo*, except in regard to the identity of the receptors that may or may not be involved. Indeed, it seems biologically improbable that cells express 10 or more functionally redundant receptors *in vivo*, as there is less negative selection against loss of function mutations in redundant genes than other genes. It seems more likely that nucleic acid sensors behave in a cell type and pathogen specific manner. Consequently, it is of interest whether HSV-1 infection of a immunologically relevant cell type, such as macrophages, is detected by the cell such that a type I IFN and/or cell death response is induced. In this thesis, I demonstrate that this is indeed that case, and that the type I IFN response to HSV-1 infection is dependent on HSV-1 replication, not merely the recognition of components of the virion envelope. I also elucidate the mechanisms by which HSV-1 dependent macrophage death occurs, showing that it involves multiple cell death pathways and is partly dependent on the HSV-1 induced type I IFN response. These observations add to the accumulating evidence indicating interaction between type I IFN and cell death pathways, as well as the complex nature of the interaction between different programmed cell death pathways themselves. These observations are in line with the complex and contradictory literature regarding type I IFN and cell death responses to HSV-1 infection. My data demonstrate that there is not an easy answer to the question 'Does HSV-1 inhibit or activate cell death signalling?'. Inhibition of one cell death response can lead to activation of others, and it is likely that many HSV-1 factors interact with the cellular components of the apoptotic, pyroptotic and necroptotic pathways. Elucidating these interactions will require extensive work. However, an important message of this thesis is that it is probably inappropriate to consider HSV-1 cell lysis a process that is not, in some way, orchestrated by programmed cell death pathways.

HIV-1 and HSV-1 co-infection has not previously been investigated. In this thesis I demonstrate that HIV-1 pre-infection has no effect on HSV-1 productive infection of macrophages. This is perhaps not surprising given that HIV-1 infection of macrophages is relatively silent in terms of cell activation. HSV-1 super-infection of HIV-1 infected cells however, leads to an increase in HIV-1 transcription. Additionally, HIV-1 virion release is not restricted by an HSV-1 dependent interferon response, as it is by recombinant type I IFN, suggesting that HSV-1 is able to protect HIV-1 release from type I IFN restriction. These data are interesting from the point of few of the observed clinical synergy between HSV-1 and HIV-1. It may be that HSV-1 increases HIV-1 transmission rates and disease, not merely by recruiting target cells to the mucosal transmission site, but by increasing or protecting HIV-1 replication within macrophages. Again, this is a difficult hypothesis to test *in vivo*.

In this thesis I show that HSV-1 can latently infect macrophages. HSV-1 latent infection of cell types other than neurons or neuron cell lines, has not previously been demonstrated. I prove latency in the methods accepted as standard by the field. These are namely the presence of HSV-1 LAT in the absence of lytic gene expression or virion production, and the ability for lytic gene expression to be reactivated. HSV-1 latency in macrophages can be reactivated by HIV-1. It is of considerable interest whether other lentivectors or viruses also have this ability. Investigation into this should also provide insights into the mechanism(s) by which reactivation is achieved.

HSV-1 latent infection of macrophages has significant implications for our understanding of HSV-1 disease and latency *in vivo*. It is difficult to assess whether tissue resident macrophages support HSV-1 latency, but this thesis does bring into question the dogma that neurons are the only reservoirs of HSV-1 during clinical latency. The implications of this for our understanding of HSV-1 disease, treatment and eradication are likely to be significant. Additionally, the model of HSV-1 latency provided by HSV-1 infection of macrophages is potentially the best model of HSV-1 latency *in vivo* to date, in that artificial interventions, such as infection in the presence of an anti-HSV-1 drug, are not necessary for the establishment of latency. HSV-1 infection of macrophages therefore potentially provides a very useful tool for investigating the mechanisms by which HSV-1 latency is established to researchers in the field.

7 References

[1] Javier Mestas and Christopher C. W. Hughes. Of mice and not men: Differences between mouse and human immunology. *The Journal of Immunology*, 172(5):2731–2738, March 2004. ISSN 0022-1767, 1550-6606. URL `http://www.jimmunol.org/content/172/5/2731`. PMID: 14978070.

[2] Sren R Paludan and Andrew G Bowie. Immune sensing of DNA. *Immunity*, 38(5):870–880, May 2013. ISSN 1097-4180. doi: 10.1016/j.immuni.2013.05.004. PMID: 23706668 PMCID: PMC3683625.

[3] Petr Broz and Denise M Monack. Newly described pattern recognition receptors team up against intracellular

pathogens. *Nature reviews. Immunology*, 13(8):551–565, August 2013. ISSN 1474-1741. doi: 10.1038/nri3479. PMID: 23846113.

[4] Mikayla R Thompson, John J Kaminski, Evelyn A Kurt-Jones, and Katherine A Fitzgerald. Pattern recognition receptors and the innate immune response to viral infection. *Viruses*, 3(6):920–940, June 2011. ISSN 1999-4915. doi: 10.3390/v3060920. PMID: 21994762 PMCID: PMC3186011.

[5] Luke C. Davies, Stephen J. Jenkins, Judith E. Allen, and Philip R. Taylor. Tissue-resident macrophages. *Nature Immunology*, 14(10):986–995, October 2013. ISSN 1529-2908. doi: 10.1038/ni.2705. URL http://www.nature.com/ni/journal/v14/n10/full/ni.2705.html.

[6] P R Taylor, L Martinez-Pomares, M Stacey, H-H Lin, G D Brown, and S Gordon. Macrophage receptors and immune recognition. *Annual review of immunology*, 23:901–944, 2005. ISSN 0732-0582. doi: 10.1146/annurev.immunol.23.021704.115816. PMID: 15771589.

[7] Gerard J Nau, Joan F L Richmond, Ann Schlesinger, Ezra G Jennings, Eric S Lander, and Richard A Young. Human macrophage activation programs induced by bacterial pathogens. *Proc Natl Acad Sci U S A*, 99(3):1503–1508, Feb 2002. doi: 10.1073/pnas.022649799. URL http://dx.doi.org/10.1073/pnas.022649799.

[8] Rose Hughes, Greg Towers, and Mahdad Noursadeghi. Innate immune interferon responses to human immunodeficiency virus-1 infection. *Rev Med Virol*, 22(4):257–266, Jul 2012. doi: 10.1002/rmv.1708. URL http://dx.doi.org/10.1002/rmv.1708.

[9] J. Tsang, B. M Chain, R. F Miller, B. L.J Webb, W. Barclay, G. J Towers, D. R Katz, and M. Noursadeghi. HIV-1 infection of macrophages is dependent on evasion of innate immunne cellular activation. *AIDS*, 23(7312): 2255–2263, 2009.

[10] Mahdad Noursadeghi, David R Katz, and Robert F Miller. Hiv-1 infection of mononuclear phagocytic cells: the case for bacterial innate immune deficiency in aids. *Lancet Infect Dis*, 6(12):794–804, Dec 2006. doi: 10.1016/S1473-3099(06)70656-9. URL http://dx.doi.org/10.1016/S1473-3099(06)70656-9.

[11] a Berra, a Rodriguez, a Heiligenhaus, B Pazos, N Van Rooijen, and C S Foster. The role of macrophages in the pathogenesis of HSV-1 induced chorioretinitis in BALB/c mice. *Investigative ophthalmology & visual science*, 35 (7):2990–8, June 1994. ISSN 0146-0404. URL http://www.ncbi.nlm.nih.gov/pubmed/7911460.

[12] D Bauer, S Mrzyk, N Van Rooijen, K P Steuhl, and a Heiligenhaus. Incidence and severity of herpetic stromal keratitis: impaired by the depletion of lymph node macrophages. *Experimental eye research*, 72(3):261–9, March 2001. ISSN 0014-4835. doi: 10.1006/exer.2000.0947. URL http://www.ncbi.nlm.nih.gov/pubmed/11180975.

[13] P Kodukula, T Liu, N V Rooijen, M J Jager, and R L Hendricks. Macrophage control of herpes simplex virus type 1 replication in the peripheral nervous system. *Journal of immunology (Baltimore, Md. : 1950)*, 162(5):2895–905, March 1999. ISSN 0022-1767. URL http://www.ncbi.nlm.nih.gov/pubmed/10072539.

[14] A.M. Foss, P.T. Vickerman, P. Mayaud, H.A. Weiss, B.M. Ramesh, S. Reza-Paul, R. Washington, J. Blanchard, S. Moses, C.M. Lowndes, M. Alary, and C.H. Watts. Modelling the interactions between herpes simplex virus type 2 and HIV: implications for the HIV epidemic in southern india. *Sexually Transmitted Infections*, 87(1):22–27, 2011. ISSN 1368-4973. doi: 10.1136/sti.2009.041699.

[15] Don C Des Jarlais, Kamyar Arasteh, Courtney McKnight, David C Perlman, Hannah L F Cooper, and Holly Hagan. HSV-2 infection as a cause of Female/Male and Racial/Ethnic disparities in HIV infection. *PloS one*, 8(6):e66874, 2013. ISSN 1932-6203. doi: 10.1371/journal.pone.0066874. PMID: 23825055 PMCID: PMC3688945.

[16] Charles A Janeway, Paul Travers, Mark Walport, Mark Shlomchik, and Mark Schlomchik Charles. The front line of host defense. In *Immunobiology: The Immune System in Health and Disease: 5th (Fifth) Edition*, page Chapter 2: Innate Immunity. Taylor & Francis, Inc., June 2001.

[17] Kasper Hoebe, Edith Janssen, and Bruce Beutler. The interface between innate and adaptive immunity. *Nature immunology*, 5(10):971–974, October 2004. ISSN 1529-2908. doi: 10.1038/ni1004-971. PMID: 15454919.

[18] Simon B. Rasmussen, Soren B. Jensen, Christoffer Nielsen, Emanuel Quartin, Hiroki Kato, Zhijian J. Chen, Robert H. Silverman, Shizuo Akira, and Soren R. Paludan. Herpes simplex virus infection is sensed by both toll-like receptors and retinoic acid-inducible gene- like receptors, which synergize to induce type i interferon production. *J Gen Virol*, 90(1):74–78, January 2009. doi: 10.1099/vir.0.005389-0.

[19] Gillian S Tomlinson, Helen Booth, Sarah J Petit, Elspeth Potton, Greg J Towers, Robert F Miller, Benjamin M Chain, and Mahdad Noursadeghi. Adherent human alveolar macrophages exhibit a transient pro-inflammatory profile that confounds responses to innate immune stimulation. *PLoS One*, 7(6):e40348, 2012. doi: 10.1371/journal.pone.0040348. URL http://dx.doi.org/10.1371/journal.pone.0040348.

[20] Emanuela Handman and Denise V R Bullen. Interaction of leishmania with the host macrophage. *Trends in parasitology*, 18(8):332–334, August 2002. ISSN 1471-4922. PMID: 12377273.

[21] C E Ibanez, R Schrier, P Ghazal, C Wiley, and J A Nelson. Human cytomegalovirus productively infects primary differentiated macrophages. *J. Virol.*, 65(12):6581–6588, December 1991.

[22] Laura J Dixon, Mark Barnes, Hui Tang, Michele T Pritchard, and Laura E Nagy. Kupffer cells in the liver. *Comprehensive Physiology*, 3(2):785–797, April 2013. ISSN 2040-4603. doi: 10.1002/cphy.c120026. PMID: 23720329.

[23] D Boche, V H Perry, and J A R Nicoll. Review: activation patterns of microglia and their identification in the human brain. *Neuropathology and applied neurobiology*, 39(1):3–18, February 2013. ISSN 1365-2990. doi: 10.1111/nan.12011. PMID: 23252647.

[24] Marc Beyer, Michael R Mallmann, Jia Xue, Andrea Staratschek-Jox, Daniela Vorholt, Wolfgang Krebs, Daniel Sommer, Jil Sander, Christina Mertens, Andrea Nino-Castro, Susanne V Schmidt, and Joachim L Schultze. High-resolution transcriptome of human macrophages. *PLoS One*, 7(9):e45466, 2012. doi: 10.1371/journal.pone.0045466. URL http://dx.doi.org/10.1371/journal.pone.0045466.

[25] David A Hume. Plenary perspective: the complexity of constitutive and inducible gene expression in mononuclear phagocytes. *J Leukoc Biol*, 92(3):433–444, Sep 2012. doi: 10.1189/jlb.0312166. URL http://dx.doi.org/10.1189/jlb.0312166.

[26] Derek C Lacey, Adrian Achuthan, Andrew J Fleetwood, Hang Dinh, John Roiniotis, Glen M Scholz, Melody W Chang, Sandra K Beckman, Andrew D Cook, and John A Hamilton. Defining gm-csf- and macrophage-csf-dependent macrophage responses by in vitro models. *J Immunol*, 188(11):5752–5765, Jun 2012. doi: 10.4049/jimmunol.1103426. URL http://dx.doi.org/10.4049/jimmunol.1103426.

[27] David A Hume. Comment on "ccr7 is critically important for migration of dendritic cells in intestinal lamina propria to mesenteric lymph nodes". *J Immunol*, 177(4):2035; author reply 2035–2035; author reply 2036, Aug 2006.

[28] Jia Xue, Susanne V Schmidt, Jil Sander, Astrid Draffehn, Wolfgang Krebs, Inga Quester, Dominic De Nardo, Trupti D Gohel, Martina Emde, Lisa Schmidleithner, Hariharasudan Ganesan, Andrea Nino-Castro, Michael R Mallmann, Larisa Labzin, Heidi Theis, Michael Kraut, Marc Beyer, Eicke Latz, Tom C Freeman, Thomas Ulas, and Joachim L Schultze. Transcriptome-based network analysis reveals a spectrum model of human macrophage activation. *Immunity*, 40(2):274–288, February 2014. ISSN 1097-4180. doi: 10.1016/j.immuni.2014.01.006. PMID: 24530056.

[29] Siamon Gordon and Philip R. Taylor. Monocyte and macrophage heterogeneity. *Nature Reviews Immunology*, 5(12):953–964, December 2005. ISSN 1474-1733. doi: 10.1038/nri1733. URL http://www.nature.com/nri/journal/v5/n12/full/nri1733.html.

[30] Frederic Geissmann, Markus G. Manz, Steffen Jung, Michael H. Sieweke, Miriam Merad, and Klaus Ley. Development of monocytes, macrophages, and dendritic cells. *Science (New York, N.Y.)*, 327(5966):656–661, February 2010. ISSN 1095-9203. doi: 10.1126/science.1178331.

[31] Christian Schulz, Elisa Gomez Perdiguero, Laurent Chorro, Heather Szabo-Rogers, Nicolas Cagnard, Katrin Kierdorf, Marco Prinz, Bishan Wu, Sten Eirik W. Jacobsen, Jeffrey W. Pollard, Jon Frampton, Karen J. Liu, and Frederic

Geissmann. A lineage of myeloid cells independent of myb and hematopoietic stem cells. *Science (New York, N.Y.)*, 336(6077):86–90, April 2012. ISSN 1095-9203. doi: 10.1126/science.1219179.

[32] Thomas A. Wynn, Ajay Chawla, and Jeffrey W. Pollard. Macrophage biology in development, homeostasis and disease. *Nature*, 496(7446):445–455, April 2013. ISSN 0028-0836. doi: 10.1038/nature12034. URL http://www.nature.com/nature/journal/v496/n7446/full/nature12034.html.

[33] John A Hamilton. Colony-stimulating factors in inflammation and autoimmunity. *Nat Rev Immunol*, 8(7):533–544, Jul 2008. doi: 10.1038/nri2356. URL http://dx.doi.org/10.1038/nri2356.

[34] Violeta Chitu and E. Richard Stanley. Colony-stimulating factor-1 in immunity and inflammation. *Curr Opin Immunol*, 18(1):39–48, Feb 2006. doi: 10.1016/j.coi.2005.11.006. URL http://dx.doi.org/10.1016/j.coi.2005.11.006.

[35] Andrew J Fleetwood, Andrew D Cook, and John A Hamilton. Functions of granulocyte-macrophage colony-stimulating factor. *Crit Rev Immunol*, 25(5):405–428, 2005.

[36] Kiyoko S Akagawa, Iwao Komuro, Hiroko Kanazawa, Toshio Yamazaki, Keiko Mochida, and Fumio Kishi. Functional heterogeneity of colony-stimulating factor-induced human monocyte-derived macrophages. *Respirology*, 11 Suppl: S32–S36, Jan 2006. doi: 10.1111/j.1440-1843.2006.00805.x. URL http://dx.doi.org/10.1111/j.1440-1843.2006.00805.x.

[37] Mandy J. McGeachy. GM-CSF: the secret weapon in the t(h)17 arsenal. *Nature Immunology*, 12(6):521–522, June 2011. ISSN 1529-2916. doi: 10.1038/ni.2044.

[38] Yufang Shi, Catherine H. Liu, Arthur I. Roberts, Jyoti Das, Guangwu Xu, Guangwen Ren, Yingyu Zhang, Liying Zhang, Zeng Rong Yuan, Hung Sheng William Tan, Gobardhan Das, and Satish Devadas. Granulocyte-macrophage colony-stimulating factor (GM-CSF) and t-cell responses: what we do and don't know. *Cell Research*, 16(2):126–133, 2006. ISSN 1001-0602. doi: 10.1038/sj.cr.7310017. URL http://www.nature.com/cr/journal/v16/n2/abs/7310017a.html.

[39] Brenna Carey and Bruce C. Trapnell. The molecular basis of pulmonary alveolar proteinosis. *Clinical immunology (Orlando, Fla.)*, 135(2):223–235, May 2010. ISSN 1521-6616. doi: 10.1016/j.clim.2010.02.017. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2866141/.

[40] Isabelle Brochriou, Seraya Maouche, Herv Durand, Vincent Braunersreuther, Gilles Le Naour, Alexei Gratchev, Fabien Koskas, Franois Mach, Julia Kzhyshkowska, and Ewa Ninio. Antagonistic regulation of macrophage phenotype by m-CSF and GM-CSF: implication in atherosclerosis. *Atherosclerosis*, 214(2):316–324, February 2011. ISSN 1879-1484. doi: 10.1016/j.atherosclerosis.2010.11.023. PMID: 21159337.

[41] Taro Kawai and Shizuo Akira. Innate immune recognition of viral infection. *Nature Immunology*, 7(2):131–137, February 2006. ISSN 1529-2908. doi: 10.1038/ni1303. URL http://www.nature.com/ni/journal/v7/n2/abs/ni1303.html.

[42] Ruslan Medzhitov. Toll-like receptors and innate immunity. *Nature Reviews Immunology*, 1(2):135–145, November 2001. ISSN 1474-1733. doi: 10.1038/35100529. URL http://www.nature.com/nri/journal/v1/n2/abs/nri1101-135a.html.

[43] Siamon Gordon. Pattern recognition receptors: Doubling up for the innate immune response. *Cell*, 111(7):927–930, December 2002. ISSN 0092-8674. doi: 10.1016/S0092-8674(02)01201-1. URL http://www.sciencedirect.com/science/article/pii/S0092867402012011.

[44] Miwa Sasai and Masahiro Yamamoto. Pathogen recognition receptors: ligands and signaling pathways by toll-like receptors. *International reviews of immunology*, 32(2):116–133, April 2013. ISSN 1563-5244. doi: 10.3109/08830185.2013.774391. PMID: 23570313.

[45] Mitsutoshi Yoneyama, Mika Kikuchi, Takashi Natsukawa, Noriaki Shinobu, Tadaatsu Imaizumi, Makoto Miyagishi, Kazunari Taira, Shizuo Akira, and Takashi Fujita. The RNA helicase RIG-I has an essential function in double-

stranded RNA-induced innate antiviral responses. *Nat Immunol*, 5(7):730–737, July 2004. ISSN 1529-2908. doi: 10.1038/ni1087.

[46] Mitsutoshi Yoneyama, Mika Kikuchi, Kanae Matsumoto, Tadaatsu Imaizumi, Makoto Miyagishi, Kazunari Taira, Eileen Foy, Yueh-Ming Loo, Michael Gale, Shizuo Akira, Shin Yonehara, Atsushi Kato, and Takashi Fujita. Shared and unique functions of the DExD/H-box helicases RIG-I, MDA5, and LGP2 in antiviral innate immunity. *Journal of Immunology (Baltimore, Md.: 1950)*, 175(5):2851–2858, September 2005. ISSN 0022-1767.

[47] Akinori Takaoka, ZhiChao Wang, Myoung Kwon Choi, Hideyuki Yanai, Hideo Negishi, Tatsuma Ban, Yan Lu, Makoto Miyagishi, Tatsuhiko Kodama, Kenya Honda, Yusuke Ohba, and Tadatsugu Taniguchi. DAI (DLM-1/ZBP1) is a cytosolic DNA sensor and an activator of innate immune response. *Nature*, 448(7152):501–505, July 2007. ISSN 0028-0836. doi: 10.1038/nature06013.

[48] Teresa Fernandes-Alnemri, Je-Wook Yu, Pinaki Datta, Jianghong Wu, and Emad S. Alnemri. AIM2 activates the inflammasome and cell death in response to cytoplasmic DNA. *Nature*, 458(7237):509–513, March 2009. ISSN 0028-0836. doi: 10.1038/nature07710.

[49] Leonie Unterholzner, Sinead E Keating, Marcin Baran, Kristy A Horan, Sren B Jensen, Shruti Sharma, Cherilyn M Sirois, Tengchuan Jin, Eicke Latz, T Sam Xiao, Katherine A Fitzgerald, Sren R Paludan, and Andrew G Bowie. IFI16 is an innate immune sensor for intracellular DNA. *Nature Immunology*, 11(11):997–1004, November 2010. ISSN 1529-2916. doi: 10.1038/ni.1932.

[50] Lijun Sun, Jiaxi Wu, Fenghe Du, Xiang Chen, and Zhijian J. Chen. Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type i interferon pathway. *Science*, 339(6121):786–791, February 2013. ISSN 0036-8075, 1095-9203. doi: 10.1126/science.1232458. URL http://www.sciencemag.org/content/339/6121/786. PMID: 23258413.

[51] Hideyuki Yanai, David Savitsky, Tomohiko Tamura, and Tadatsugu Taniguchi. Regulation of the cytosolic DNA-sensing system in innate immunity: a current view. *Current Opinion in Immunology*, 21(1):17–22, February 2009. ISSN 0952-7915. doi: 10.1016/j.coi.2009.01.005.

[52] Alina Baum and Adolfo Garca-Sastre. Induction of type i interferon by rna viruses: cellular receptors and their substrates. *Amino Acids*, 38(5):1283–1299, May 2010. doi: 10.1007/s00726-009-0374-0. URL http://dx.doi.org/10.1007/s00726-009-0374-0.

[53] Qinmiao Sun, Lijun Sun, Hong-Hsing Liu, Xiang Chen, Rashu B Seth, James Forman, and Zhijian J Chen. The specific and essential role of MAVS in antiviral innate immune responses. *Immunity*, 24(5):633–642, May 2006. ISSN 1074-7613. doi: 10.1016/j.immuni.2006.04.004.

[54] Daniel B Stetson and Ruslan Medzhitov. Recognition of cytosolic DNA activates an IRF3-dependent innate immune response. *Immunity*, 24(1):93–103, January 2006. ISSN 1074-7613. doi: 10.1016/j.immuni.2005.12.003.

[55] Kenya Honda and Tadatsugu Taniguchi. IRFs: master regulators of signalling by toll-like receptors and cytosolic pattern-recognition receptors. *Nature Reviews. Immunology*, 6(9):644–658, September 2006. ISSN 1474-1733. doi: 10.1038/nri1900.

[56] Xiao-Dong Li, Jiaxi Wu, Daxing Gao, Hua Wang, Lijun Sun, and Zhijian J Chen. Pivotal roles of cGAS-cGAMP signaling in antiviral defense and immune adjuvant effects. *Science (New York, N.Y.)*, 341(6152):1390–1394, September 2013. ISSN 1095-9203. doi: 10.1126/science.1244040. PMID: 23989956 PMCID: PMC3863637.

[57] Xin Wang, Shu-Mei Huong, Marie L Chiu, Nancy Raab-Traub, and Eng-Shang Huang. Epidermal growth factor receptor is a cellular receptor for human cytomegalovirus. *Nature*, 424(6947):456–461, July 2003. ISSN 1476-4687. doi: 10.1038/nature01818.

[58] John Hiscott. Convergence of the NF-kappaB and IRF pathways in the regulation of the innate antiviral response. *Cytokine & Growth Factor Reviews*, 18(5-6):483–490, December 2007. ISSN 1359-6101. doi: 10.1016/j.cytogfr.2007.06.002.

[59] Siddharth Balachandran and Amer A. Beg. Defining emerging roles for NF-?b in antivirus responses: Revisiting the interferon- enhanceosome paradigm. *PLoS Pathogens*, 7(10), October 2011. ISSN 1553-7366. doi: 10.1371/journal.ppat.1002165. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3192840/.

[60] George D Kalliolias and Lionel B Ivashkiv. Overview of the biology of type i interferons. *Arthritis Res Ther*, 12 Suppl 1:S1, 2010. doi: 10.1186/ar2881. URL http://dx.doi.org/10.1186/ar2881.

[61] Nina Ank, Hans West, and Sren R Paludan. Ifn-lambda: novel antiviral cytokines. *J Interferon Cytokine Res*, 26 (6):373–379, Jun 2006. doi: 10.1089/jir.2006.26.373. URL http://dx.doi.org/10.1089/jir.2006.26.373.

[62] Leonidas C. Platanias. Mechanisms of type-i- and type-II-interferon-mediated signalling. *Nature Reviews Immunology*, 5(5):375–386, May 2005. ISSN 1474-1733. doi: 10.1038/nri1604. URL http://www.nature.com/nri/journal/v5/n5/full/nri1604.html.

[63] Cludio A. Bonjardim, Paulo C. P. Ferreira, and Erna G. Kroon. Interferons: Signaling, antiviral and viral evasion. *Immunology Letters*, 122(1):1–11, January 2009. ISSN 0165-2478. doi: 10.1016/j.imlet.2008.11.002. URL http://www.sciencedirect.com/science/article/pii/S0165247808002484.

[64] M.M. Song and K. Shuai. The suppressor of cytokine signaling (SOCS) 1 and SOCS3 but not SOCS2 proteins inhibit interferon-mediated antiviral and antiproliferative activities. *Journal of Biological Chemistry*, 273(52):35056–35062, 1998. ISSN 0021-9258. doi: 10.1074/jbc.273.52.35056.

[65] A. Yoshimura, T. Naka, and M. Kubo. SOCS proteins, cytokine signalling and immune regulation. *Nature Reviews Immunology*, 7(6):454–465, 2007. ISSN 1474-1733. doi: 10.1038/nri2093.

[66] Ben A. Croker, Hiu Kiu, and Sandra E. Nicholson. SOCS regulation of the JAK/STAT signalling pathway. *Seminars in cell & developmental biology*, 19(4):414–422, August 2008. ISSN 1084-9521. doi: 10.1016/j.semcdb.2008.07.010. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2597703/. PMID: 18708154 PMCID: PMC2597703.

[67] J A Sonnabend, I M Kerr, and E M Martin. Development of the antiviral state in response to interferon. *The Journal of general physiology*, 56(1):172–183, July 1970. ISSN 0022-1295. PMID: 19873665 PMCID: PMC2225884.

[68] D E Levy and A Garca-Sastre. The virus battles: IFN induction of the antiviral state and mechanisms of viral evasion. *Cytokine & growth factor reviews*, 12(2-3):143–156, September 2001. ISSN 1359-6101. PMID: 11325598.

[69] Sonali Joshi, Surinder Kaur, Barbara Kroczynska, and Leonidas C Platanias. Mechanisms of mrna translation of interferon stimulated genes. *Cytokine*, 52(1-2):123–127, 2010. doi: 10.1016/j.cyto.2010.03.019. URL http://dx.doi.org/10.1016/j.cyto.2010.03.019.

[70] G. C. Sen, H. Taira, and P. Lengyel. Interferon, double-stranded RNA, and protein phosphorylation. characteristics of a double-stranded RNA-activated protein kinase system partially purified from interferon treated ehrlich ascites tumor cells. *Journal of Biological Chemistry*, 253(17):5915–5921, September 1978. ISSN 0021-9258, 1083-351X. URL http://www.jbc.org/content/253/17/5915. PMID: 210162.

[71] M J Clemens. PKR–a protein kinase regulated by double-stranded RNA. *The international journal of biochemistry & cell biology*, 29(7):945–949, July 1997. ISSN 1357-2725. PMID: 9375375.

[72] M. A. Garca, J. Gil, I. Ventoso, S. Guerra, E. Domingo, C. Rivas, and M. Esteban. Impact of protein kinase pkr in cell biology: from antiviral to antiproliferative action. *Microbiol Mol Biol Rev*, 70(4):1032–1060, Dec 2006. doi: 10.1128/MMBR.00027-06. URL http://dx.doi.org/10.1128/MMBR.00027-06.

[73] B. R. Williams. Pkr; a sentinel kinase for cellular stress. *Oncogene*, 18(45):6112–6120, Nov 1999. doi: 10.1038/sj.onc.1203127. URL http://dx.doi.org/10.1038/sj.onc.1203127.

[74] Otto Haller and Georg Kochs. Interferon-induced mx proteins: Dynamin-like GTPases with antiviral activity. *Traffic*, 3(10):710–717, October 2002. ISSN 1600-0854. doi: 10.1034/j.1600-0854.2002.31003.x. URL http://onlinelibrary.wiley.com/doi/10.1034/j.1600-0854.2002.31003.x/abstract.

[75] Volker Fensterl and Ganes C Sen. The ISG56/IFIT1 gene family. *Journal of interferon & cytokine research: the*

official journal of the International Society for Interferon and Cytokine Research, 31(1):71–78, January 2011. ISSN 1557-7465. doi: 10.1089/jir.2010.0101. PMID: 20950130 PMCID: PMC3021354.

[76] K. M. Vattem, K. A. Staschke, and R. C. Wek. Mechanism of activation of the double-stranded-RNA-dependent protein kinase, PKR: role of dimerization and cellular localization in the stimulation of PKR phosphorylation of eukaryotic initiation factor-2 (eIF2). *European journal of biochemistry / FEBS*, 268(13):3674–3684, July 2001. ISSN 0014-2956.

[77] S. Wu and R. J. Kaufman. A model for the double-stranded RNA (dsRNA)-dependent dimerization and activation of the dsRNA-activated protein kinase PKR. *The Journal of Biological Chemistry*, 272(2):1291–1296, January 1997. ISSN 0021-9258.

[78] S. P. Srivastava, K. U. Kumar, and R. J. Kaufman. Phosphorylation of eukaryotic translation initiation factor 2 mediates apoptosis in response to activation of the double-stranded RNA-dependent protein kinase. *The Journal of Biological Chemistry*, 273(4):2416–2423, January 1998. ISSN 0021-9258.

[79] Samantha Burugu, Acha Daher, Eliane F. Meurs, and Anne Gatignol. HIV-1 translation and its regulation by cellular factors PKR and PACT. *Virus Research*, July 2014. ISSN 1872-7492. doi: 10.1016/j.virusres.2014.07.014.

[80] Gregory A. Peters, David Khoo, Ian Mohr, and Ganes C. Sen. Inhibition of PACT-mediated activation of PKR by the herpes simplex virus type 1 us11 protein. *Journal of Virology*, 76(21):11054–11064, November 2002. ISSN 0022-538X. doi: 10.1128/JVI.76.21.11054-11064.2002. URL `http://www.ncbi.nlm.nih.gov/pmc/articles/PMC136652/`.

[81] B. Dong and R. H. Silverman. A bipartite model of 2-5a-dependent RNase l. *The Journal of Biological Chemistry*, 272(35):22236–22242, August 1997. ISSN 0021-9258.

[82] M. J. Clemens and B. R. Williams. Inhibition of cell-free protein synthesis by pppA2'p5'a2'p5'a: a novel oligonucleotide synthesized by interferon-treated l cell extracts. *Cell*, 13(3):565–572, March 1978. ISSN 0092-8674.

[83] Veit Hornung, Rune Hartmann, Andrea Ablasser, and Karl-Peter Hopfner. OAS proteins and cGAS: unifying concepts in sensing and responding to cytosolic nucleic acids. *Nature Reviews Immunology*, 14(8):521–528, August 2014. ISSN 1474-1733. doi: 10.1038/nri3719. URL `http://www.nature.com/nri/journal/v14/n8/full/nri3719.html`.

[84] J. C. Castelli, B. A. Hassel, A. Maran, J. Paranjape, J. A. Hewitt, X. L. Li, Y. T. Hsu, R. H. Silverman, and R. J. Youle. The role of 2'-5' oligoadenylate-activated ribonuclease l in apoptosis. *Cell Death and Differentiation*, 5(4): 313–320, April 1998. ISSN 1350-9047. doi: 10.1038/sj.cdd.4400352.

[85] Ricardo Sanchez and Ian Mohr. Inhibition of cellular 2?-5? oligoadenylate synthetase by the herpes simplex virus type 1 us11 protein. *Journal of Virology*, 81(7):3455–3464, April 2007. ISSN 0022-538X. doi: 10.1128/JVI.02520-06. URL `http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1866071/`.

[86] Otto Haller, Peter Staeheli, and Georg Kochs. Interferon-induced mx proteins in antiviral host defense. *Biochimie*, 89(6-7):812–818, July 2007. ISSN 0300-9084. doi: 10.1016/j.biochi.2007.04.015.

[87] Caroline Goujon and Michael H Malim. Characterization of the alpha interferon-induced postentry block to HIV-1 infection in primary human macrophages and t cells. *Journal of Virology*, 84(18):9254–9266, September 2010. ISSN 1098-5514. doi: 10.1128/JVI.00854-10.

[88] Matthew Stremlau, Christopher M Owens, Michel J Perron, Michael Kiessling, Patrick Autissier, and Joseph Sodroski. The cytoplasmic body component trim5alpha restricts hiv-1 infection in old world monkeys. *Nature*, 427 (6977):848–853, Feb 2004. doi: 10.1038/nature02343. URL `http://dx.doi.org/10.1038/nature02343`.

[89] Stuart Neil and Paul Bieniasz. Human immunodeficiency virus, restriction factors, and interferon. *J Interferon Cytokine Res*, 29(9):569–580, Sep 2009. doi: 10.1089/jir.2009.0077. URL `http://dx.doi.org/10.1089/jir.2009.0077`.

[90] Adam J Fletcher and Greg J Towers. Inhibition of retroviral replication by members of the TRIM protein family. *Current topics in microbiology and immunology*, 371:29–66, 2013. ISSN 0070-217X. doi: 10.1007/978-3-642-37765-5_2. PMID: 23686231.

[91] Caroline Blondeau, Annegret Pelchen-Matthews, Petra Mlcochova, Mark Marsh, Richard S B Milne, and Greg J Towers. Tetherin restricts herpes simplex virus 1 and is antagonized by glycoprotein m. *Journal of virology*, 87 (24):13124–13133, December 2013. ISSN 1098-5514. doi: 10.1128/JVI.02250-13. PMID: 24067975 PMCID: PMC3838283.

[92] Helen L Zenner, Rui Mauricio, George Banting, and Colin M Crump. Herpes simplex virus 1 counteracts tetherin restriction via its virion host shutoff activity. *Journal of virology*, 87(24):13115–13123, December 2013. ISSN 1098-5514. doi: 10.1128/JVI.02167-13. PMID: 24067977 PMCID: PMC3838292.

[93] Peter Gee, Yoshinori Ando, Hiroko Kitayama, Seiji P. Yamamoto, Yuka Kanemura, Hirotaka Ebina, Yasushi Kawaguchi, and Yoshio Koyanagi. APOBEC1-mediated editing and attenuation of herpes simplex virus 1 DNA indicate that neurons have an antiviral role during herpes simplex encephalitis . *Journal of Virology*, 85(19):9726–9736, October 2011. ISSN 0022-538X. doi: 10.1128/JVI.05288-11. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3196441/.

[94] Rodolphe Suspne, Marie-Ming Aynaud, Stefanie Koch, David Pasdeloup, Marc Labetoulle, Barbara Gaertner, Jean-Pierre Vartanian, Andreas Meyerhans, and Simon Wain-Hobson. Genetic editing of herpes simplex virus 1 and epstein-barr herpesvirus genomes by human APOBEC3 cytidine deaminases in culture and in vivo. *Journal of Virology*, 85(15):7594–7602, August 2011. ISSN 1098-5514. doi: 10.1128/JVI.00290-11.

[95] Belete A. Desimmie, Krista A. Delviks-Frankenberrry, Ryan C. Burdick, DongFei Qi, Taisuke Izumi, and Vinay K. Pathak. Multiple APOBEC3 restriction factors for HIV-1 and one vif to rule them all. *Journal of Molecular Biology*, 426(6):1220–1245, March 2014. ISSN 1089-8638. doi: 10.1016/j.jmb.2013.10.033.

[96] Ann M. Sheehy, Nathan C. Gaddis, Jonathan D. Choi, and Michael H. Malim. Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral vif protein. *Nature*, 418(6898):646–650, August 2002. ISSN 0028-0836. doi: 10.1038/nature00939.

[97] Hui Zhang, Bin Yang, Roger J Pomerantz, Chune Zhang, Shyamala C Arunachalam, and Ling Gao. The cytidine deaminase CEM15 induces hypermutation in newly synthesized HIV-1 DNA. *Nature*, 424(6944):94–98, July 2003. ISSN 1476-4687. doi: 10.1038/nature01707.

[98] Kim Stopak, Carlos de Noronha, Wes Yonemoto, and Warner C. Greene. HIV-1 vif blocks the antiviral activity of APOBEC3g by impairing both its translation and intracellular stability. *Molecular Cell*, 12(3):591–601, September 2003. ISSN 1097-2765.

[99] Jennifer L. Fribourgh, Henry C. Nguyen, Leslie S. Wolfe, David C. Dewitt, Wenyan Zhang, Xiao-Fang Yu, Elizabeth Rhoades, and Yong Xiong. Core binding factor beta plays a critical role by facilitating the assembly of the vif-cullin 5 e3 ubiquitin ligase. *Journal of Virology*, 88(6):3309–3319, March 2014. ISSN 1098-5514. doi: 10.1128/JVI.03824-13.

[100] Xianghui Yu, Yunkai Yu, Bindong Liu, Kun Luo, Wei Kong, Panyong Mao, and Xiao-Fang Yu. Induction of APOBEC3g ubiquitination and degradation by an HIV-1 vif-cul5-SCF complex. *Science*, 302(5647):1056–1060, November 2003. ISSN 0036-8075, 1095-9203. doi: 10.1126/science.1089591. URL http://www.sciencemag.org/content/302/5647/1056.

[101] Leslie S. Wolfe, Bradford J. Stanley, Chang Liu, William K. Eliason, and Yong Xiong. Dissection of the HIV vif interaction with human e3 ubiquitin ligase. *Journal of Virology*, 84(14):7135–7139, July 2010. ISSN 1098-5514. doi: 10.1128/JVI.00031-10.

[102] Holly A. Sadler, Mark D. Stenglein, Reuben S. Harris, and Louis M. Mansky. APOBEC3g contributes to HIV-1 variation through sublethal mutagenesis. *Journal of Virology*, 84(14):7396–7404, July 2010. ISSN 0022-538X, 1098-5514. doi: 10.1128/JVI.00056-10. URL http://jvi.asm.org/content/84/14/7396.

[103] Galle Mercenne, Serena Bernacchi, Delphine Richer, Guillaume Bec, Simon Henriet, Jean-Christophe Paillart, and Roland Marquet. HIV-1 vif binds to APOBEC3g mRNA and inhibits its translation. *Nucleic Acids Research*, 38(2):633–646, January 2010. ISSN 0305-1048, 1362-4962. doi: 10.1093/nar/gkp1009. URL `http://nar.oxfordjournals.org/content/38/2/633`.

[104] Yuqing Feng, Robin P. Love, and Linda Chelico. HIV-1 viral infectivity factor (vif) alters processive single-stranded DNA scanning of the retroviral restriction factor APOBEC3g. *Journal of Biological Chemistry*, 288(9):6083–6094, March 2013. ISSN 0021-9258, 1083-351X. doi: 10.1074/jbc.M112.421875. URL `http://www.jbc.org/content/288/9/6083`.

[105] Stuart J D Neil, Trinity Zang, and Paul D Bieniasz. Tetherin inhibits retrovirus release and is antagonized by HIV-1 vpu. *Nature*, 451(7177):425–430, January 2008. ISSN 1476-4687. doi: 10.1038/nature06553.

[106] Matthew W. McNatt, Trinity Zang, and Paul D. Bieniasz. Vpu binds directly to tetherin and displaces it from nascent virions. *PLoS Pathog*, 9(4):e1003299, April 2013. doi: 10.1371/journal.ppat.1003299. URL `http://dx.doi.org/10.1371/journal.ppat.1003299`.

[107] Bastien Mangeat, Gustavo Gers-Huber, Martin Lehmann, Madeleine Zufferey, Jeremy Luban, and Vincent Piguet. HIV-1 vpu neutralizes the antiviral factor Tetherin/BST-2 by binding it and directing its beta-TrCP2-Dependent degradation. *PLoS Pathog*, 5(9):e1000574, September 2009. doi: 10.1371/journal.ppat.1000574. URL `http://dx.doi.org/10.1371/journal.ppat.1000574`.

[108] Michael J. de Veer, Michelle Holko, Mathias Frevel, Eldon Walker, Sandy Der, Jayashree M. Paranjape, Robert H. Silverman, and Bryan R. G. Williams. Functional classification of interferon-stimulated genes identified using microarrays. *Journal of Leukocyte Biology*, 69(6):912–920, June 2001. ISSN 0741-5400, 1938-3673. URL `http://www.jleukbio.org/content/69/6/912`.

[109] Paola Rizza, Franca Moretti, and Filippo Belardelli. Recent advances on the immunomodulatory effects of IFN-alpha: implications for cancer immunotherapy and autoimmunity. *Autoimmunity*, 43(3):204–209, April 2010. ISSN 1607-842X. doi: 10.3109/08916930903510880. PMID: 20187707.

[110] Thomas B Thornley, Nancy E Phillips, Britte C Beaudette-Zlatanova, Thomas G Markees, Kapil Bahl, Michael A Brehm, Leonard D Shultz, Evelyn A Kurt-Jones, John P Mordes, Raymond M Welsh, Aldo A Rossini, and Dale L Greiner. Type 1 ifn mediates cross-talk between innate and adaptive immunity that abrogates transplantation tolerance. *J Immunol*, 179(10):6620–6629, Nov 2007.

[111] Koji Kono, Flavio Salazar-Onfray, Max Petersson, Johan Hansson, Giuseppe Masucci, Ken Wasserman, Tsutomu Nakazawa, Paul Anderson, and Rolf Kiessling. Hydrogen peroxide secreted by tumor-derived macrophages down-modulates signal-transducing zeta molecules and inhibits tumor-specific t cell-and natural killer cell-mediated cytotoxicity. *European Journal of Immunology*, 26(6):1308–1313, June 1996. ISSN 1521-4141. doi: 10.1002/eji.1830260620. URL `http://onlinelibrary.wiley.com/doi/10.1002/eji.1830260620/abstract`.

[112] Georges Herbein, Ulrich Mahlknecht, Franak Batliwalla, Peter Gregersen, Todd Pappas, John Butler, William A. O'Brien, and Eric Verdin. Apoptosis of CD8+ t cells is mediated by macrophages through interaction of HIV gp120 with chemokine receptor CXCR4. *Nature*, 395(6698):189–194, September 1998. ISSN 0028-0836. doi: 10.1038/26026. URL `http://www.nature.com/nature/journal/v395/n6698/abs/395189a0.html`.

[113] Helena Cucak, Ulf Yrlid, Boris Reizis, Ulrich Kalinke, and Bengt Johansson-Lindbom. Type i interferon signaling in dendritic cells stimulates the development of lymph-node-resident t follicular helper cells. *Immunity*, 31(3):491–501, Sep 2009. doi: 10.1016/j.immuni.2009.07.005. URL `http://dx.doi.org/10.1016/j.immuni.2009.07.005`.

[114] A. Le Bon, G. Schiavoni, G. D'Agostino, I. Gresser, F. Belardelli, and D. F. Tough. Type i interferons potently enhance humoral immunity and can promote isotype switching by stimulating dendritic cells in vivo. *Immunity*, 14 (4):461–470, Apr 2001.

[115] Diana L Brassard, Michael J Grace, and Ronald W Bordens. Interferon-alpha as an immunotherapeutic protein. *J Leukoc Biol*, 71(4):565–581, Apr 2002.

[116] Agnes Le Bon, Clare Thompson, Elisabeth Kamphuis, Vanessa Durand, Cornelia Rossmann, Ulrich Kalinke, and David F Tough. Cutting edge: enhancement of antibody responses through direct stimulation of b and t cells by type i ifn. *J Immunol*, 176(4):2074–2078, Feb 2006.

[117] Gaetan Jego, A. Karolina Palucka, Jean-Philippe Blanck, Cecile Chalouni, Virginia Pascual, and Jacques Banchereau. Plasmacytoid dendritic cells induce plasma cell differentiation through type i interferon and interleukin 6. *Immunity*, 19(2):225–234, Aug 2003.

[118] Petr Broz and Denise M Monack. Molecular mechanisms of inflammasome activation during microbial infections. *Immunological reviews*, 243(1):174–190, September 2011. ISSN 1600-065X. doi: 10.1111/j.1600-065X.2011.01041.x. PMID: 21884176 PMCID: PMC3170129.

[119] K Labbe and M Saleh. Cell death in the host response to infection. *Cell death and differentiation*, 15(9):1339–1349, September 2008. ISSN 1350-9047. doi: 10.1038/cdd.2008.91. PMID: 18566602.

[120] G N Barber. Host defense, viruses and apoptosis. *Cell death and differentiation*, 8(2):113–126, February 2001. ISSN 1350-9047. doi: 10.1038/sj.cdd.4400823. PMID: 11313713.

[121] Dyana K. Dalton, Laura Haynes, Cong-Qiu Chu, Susan L. Swain, and Susan Wittmer. Interferon eliminates responding cd4 t cells during mycobacterial infection by inducing apoptosis of activated cd4 t cells. *The Journal of Experimental Medicine*, 192(1):117–122, July 2000. ISSN 0022-1007, 1540-9538. doi: 10.1084/jem.192.1.117. URL http://jem.rupress.org/content/192/1/117. PMID: 10880532.

[122] Elizabeth Turpin, Kimberly Luke, Jeremy Jones, Terrence Tumpey, Kouacou Konan, and Stacey Schultz-Cherry. Influenza virus infection increases p53 activity: Role of p53 in cell death and viral replication. *Journal of Virology*, 79(14):8802–8811, July 2005. ISSN 0022-538X, 1098-5514. doi: 10.1128/JVI.79.14.8802-8811.2005. URL http://jvi.asm.org/content/79/14/8802. PMID: 15994774.

[123] E R James and D R Green. Infection and the origins of apoptosis. *Cell death and differentiation*, 9(4):355–357, April 2002. ISSN 1350-9047. doi: 10.1038/sj/cdd/4400986. PMID: 11965487.

[124] Yan Shi, James E. Evans, and Kenneth L. Rock. Molecular identification of a danger signal that alerts the immune system to dying cells. *Nature*, 425(6957):516–521, October 2003. ISSN 0028-0836. doi: 10.1038/nature01991. URL http://www.nature.com/nature/journal/v425/n6957/abs/nature01991.html.

[125] Hajime Kono and Kenneth L. Rock. How dying cells alert the immune system to danger. *Nature Reviews Immunology*, 8(4):279–289, April 2008. ISSN 1474-1733. doi: 10.1038/nri2215. URL http://www.nature.com/nri/journal/v8/n4/abs/nri2215.html.

[126] Sonja M Best. Viral subversion of apoptotic enzymes: escape from death row. *Annual review of microbiology*, 62:171–192, 2008. ISSN 0066-4227. doi: 10.1146/annurev.micro.62.081307.163009. PMID: 18729734 PMCID: PMC2562643.

[127] Mohamed Lamkanfi and Vishva M Dixit. Manipulation of host cell death pathways during microbial infections. *Cell host & microbe*, 8(1):44–54, July 2010. ISSN 1934-6069. doi: 10.1016/j.chom.2010.06.007. PMID: 20638641.

[128] Jason W Upton, William J Kaiser, and Edward S Mocarski. Virus inhibition of RIP3-dependent necrosis. *Cell host & microbe*, 7(4):302–313, April 2010. ISSN 1934-6069. doi: 10.1016/j.chom.2010.03.006. PMID: 20413098.

[129] Germain Gillet and Gilbert Brun. Viral inhibition of apoptosis. *Trends in Microbiology*, 4(8):312–317, August 1996. ISSN 0966-842X. doi: 10.1016/0966-842X(96)10047-0. URL http://www.sciencedirect.com/science/article/pii/0966842X96100470.

[130] Stewart Hay and George Kannourakis. A time to kill: viral manipulation of the cell death program. *Journal of General Virology*, 83(7):1547–1564, July 2002. ISSN 0022-1317, 1465-2099. URL http://vir.sgmjournals.org/content/83/7/1547. PMID: 12075073.

[131] J Gil, M Bermejo, and J Alcam. HIV and apoptosis: a complex interaction between cell death and virus survival. *Progress in molecular and subcellular biology*, 36:117–149, 2004. ISSN 0079-6484. PMID: 15171610.

[132] Tom Berghe, Andreas Linkermann, Sandrine Jouan-Lanhouet, Henning Walczak, and Peter Vandenabeele. Regulated necrosis: the expanding network of non-apoptotic cell death pathways. *Nature reviews. Molecular cell biology*, 15(2):135–147, February 2014. ISSN 1471-0080. doi: 10.1038/nrm3737. PMID: 24452471.

[133] Lorenzo Galluzzi, Oliver Kepp, Stefan Krautwald, Guido Kroemer, and Andreas Linkermann. Molecular mechanisms of regulated necrosis. *Seminars in cell & developmental biology*, February 2014. ISSN 1096-3634. doi: 10.1016/j.semcdb.2014.02.006. PMID: 24582829.

[134] L Galluzzi, I Vitale, J M Abrams, E S Alnemri, E H Baehrecke, M V Blagosklonny, T M Dawson, V L Dawson, W S El-Deiry, S Fulda, E Gottlieb, D R Green, M O Hengartner, O Kepp, R A Knight, S Kumar, S A Lipton, X Lu, F Madeo, W Malorni, P Mehlen, G Nuez, M E Peter, M Piacentini, D C Rubinsztein, Y Shi, H-U Simon, P Vandenabeele, E White, J Yuan, B Zhivotovsky, G Melino, and G Kroemer. Molecular definitions of cell death subroutines: recommendations of the nomenclature committee on cell death 2012. *Cell death and differentiation*, 19 (1):107–120, January 2012. ISSN 1476-5403. doi: 10.1038/cdd.2011.96. PMID: 21760595 PMCID: PMC3252826.

[135] Jennifer V Lu and Craig M Walsh. Programmed necrosis and autophagy in immune function. *Immunological reviews*, 249(1):205–217, September 2012. ISSN 1600-065X. doi: 10.1111/j.1600-065X.2012.01147.x. PMID: 22889224.

[136] G Kroemer, L Galluzzi, P Vandenabeele, J Abrams, ES Alnemri, EH Baehrecke, MV Blagosklonny, WS El-Deiry, P Golstein, DR Green, M Hengartner, RA Knight, S Kumar, SA Lipton, W Malorni, G Nunez, ME Peter, J Tschopp, J Yuan, M Piacentini, B Zhivotovsky, and G Melino. Classification of cell death. *Cell death and differentiation*, 16(1):3–11, January 2009. ISSN 1350-9047. doi: 10.1038/cdd.2008.150. URL `http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2744427/`. PMID: 18846107 PMCID: PMC2744427.

[137] S Cruchten and W Van Den Broeck. Morphological and biochemical aspects of apoptosis, oncosis and necrosis. *Anatomia, histologia, embryologia*, 31(4):214–223, August 2002. ISSN 0340-2096. PMID: 12196263.

[138] Edward A. Miao, Jayant V. Rajan, and Alan Aderem. Caspase-1-induced pyroptotic cell death. *Immunol Rev*, 243(1): 206–214, Sep 2011. doi: 10.1111/j.1600-065X.2011.01044.x. URL `http://dx.doi.org/10.1111/j.1600-065X.2011.01044.x`.

[139] A. Zychlinsky, M. C. Prevost, and P. J. Sansonetti. Shigella flexneri induces apoptosis in infected macrophages. *Nature*, 358(6382):167–169, Jul 1992. doi: 10.1038/358167a0. URL `http://dx.doi.org/10.1038/358167a0`.

[140] L. M. Chen, K. Kaniga, and J. E. Galn. Salmonella spp. are cytotoxic for cultured macrophages. *Mol Microbiol*, 21(5):1101–1115, Sep 1996.

[141] Tessa Bergsbaken, Susan L. Fink, and Brad T. Cookson. Pyroptosis: host cell death and inflammation. *Nature Reviews Microbiology*, 7(2):99–109, February 2009. ISSN 1740-1526. doi: 10.1038/nrmicro2070. URL `http://www.nature.com/nrmicro/journal/v7/n2/abs/nrmicro2070.html`.

[142] M O Hengartner. The biochemistry of apoptosis. *Nature*, 407(6805):770–776, October 2000. ISSN 0028-0836. doi: 10.1038/35037710. PMID: 11048727.

[143] Jiaxi Wu, Lijun Sun, Xiang Chen, Fenghe Du, Heping Shi, Chuo Chen, and Zhijian J. Chen. Cyclic GMP-AMP is an endogenous second messenger in innate immune signaling by cytosolic DNA. *Science*, 339(6121):826–830, February 2013. ISSN 0036-8075, 1095-9203. doi: 10.1126/science.1229963. URL `http://www.sciencemag.org/content/339/6121/826`. PMID: 23258412.

[144] C. Garrido, L. Galluzzi, M. Brunet, P. E. Puig, C. Didelot, and G. Kroemer. Mechanisms of cytochrome c release from mitochondria. *Cell Death and Differentiation*, 13(9):1423–1433, September 2006. ISSN 1350-9047. doi: 10.1038/sj.cdd.4401950.

[145] Young Sik Cho, Sreerupa Challa, David Moquin, Ryan Genga, Tathagat Dutta Ray, Melissa Guildford, and Francis Ka-Ming Chan. Phosphorylation-driven assembly of the RIP1-RIP3 complex regulates programmed necrosis and virus-induced inflammation. *Cell*, 137(6):1112–1123, June 2009. ISSN 1097-4172. doi: 10.1016/j.cell.2009.05.037. PMID: 19524513 PMCID: PMC2727676.

[146] Zsuzsanna A. Dunai, Gergely Imre, Gabor Barna, Tamas Korcsmaros, Istvan Petak, Pal I. Bauer, and Rudolf Mihalik. Staurosporine induces necroptotic cell death under caspase-compromised conditions in u937 cells. *PLoS ONE*, 7(7):e41945, July 2012. doi: 10.1371/journal.pone.0041945. URL http://dx.doi.org/10.1371/journal.pone.0041945.

[147] Hirofumi Sawai. Differential effects of caspase inhibitors on TNF-induced necroptosis. *Biochemical and biophysical research communications*, 432(3):451–455, March 2013. ISSN 1090-2104. doi: 10.1016/j.bbrc.2013.01.126. PMID: 23410748.

[148] Andrew Oberst, Christopher P Dillon, Ricardo Weinlich, Laura L McCormick, Patrick Fitzgerald, Cristina Pop, Razq Hakem, Guy S Salvesen, and Douglas R Green. Catalytic activity of the caspase-8-FLIP(L) complex inhibits RIPK3-dependent necrosis. *Nature*, 471(7338):363–367, March 2011. ISSN 1476-4687. doi: 10.1038/nature09852. PMID: 21368763 PMCID: PMC3077893.

[149] William J Kaiser, Jason W Upton, Alyssa B Long, Devon Livingston-Rosanoff, Lisa P Daley-Bauer, Razqallah Hakem, Tamara Caspary, and Edward S Mocarski. RIP3 mediates the embryonic lethality of caspase-8-deficient mice. *Nature*, 471(7338):368–372, March 2011. ISSN 1476-4687. doi: 10.1038/nature09857. PMID: 21368762 PMCID: PMC3060292.

[150] Tessa Bergsbaken and Brad T. Cookson. Macrophage activation redirects yersinia-infected host cell death from apoptosis to caspase-1-dependent pyroptosis. *PLoS pathogens*, 3(11):e161, November 2007. ISSN 1553-7374. doi: 10.1371/journal.ppat.0030161.

[151] Mark Fields, Mei Zheng, Ming Zhang, and Sally S Atherton. Tumor necrosis factor alpha and macrophages in the brain of herpes simplex virus type 1-infected BALB/c mice. *Journal of neurovirology*, 12(6):443–55, December 2006. ISSN 1355-0284. doi: 10.1080/13550280601039030. URL http://www.ncbi.nlm.nih.gov/pubmed/17162660.

[152] Bernard Roizman. The family herpesviridae: General description, taxonomy, and classification. In Bernard Roizman, editor, *The Herpesviruses*, The Viruses, pages 1–23. Springer US, January 1982. ISBN 978-1-4684-4165-9, 978-1-4684-4163-5. URL http://link.springer.com/chapter/10.1007/978-1-4684-4163-5_1.

[153] Andrew J. Davison. Overview of classification. In Ann Arvin, Gabriella Campadelli-Fiume, Edward Mocarski, Patrick S. Moore, Bernard Roizman, Richard Whitley, and Koichi Yamanishi, editors, *Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis*. Cambridge University Press, Cambridge, 2007. ISBN 9780521827140. URL http://www.ncbi.nlm.nih.gov/books/NBK47406/. PMID: 21348096.

[154] Charlotte Mahiet, Ayla Ergani, Nicolas Huot, Nicolas Alende, Ahmed Azough, Fabrice Salvaire, Aaron Bensimon, Emmanuel Conseiller, Simon Wain-Hobson, Marc Labetoulle, and Sbastien Barradeau. Structural variability of the herpes simplex virus 1 genome in vitro and in vivo. *Journal of virology*, 86(16):8592–8601, August 2012. ISSN 1098-5514. doi: 10.1128/JVI.00223-12. PMID: 22674981 PMCID: PMC3421737.

[155] Domenico Tortorella, Benjamin E. Gewurz, Margo H. Furman, Danny J. Schust, and Hidde L. Ploegh. Viral subversion of the immune system. *Annual Review of Immunology*, 18(1):861–926, 2000. doi: 10.1146/annurev.immunol.18.1.861. URL http://www.annualreviews.org/doi/abs/10.1146/annurev.immunol.18.1.861. PMID: 10837078.

[156] Matthew Reeves and John Sinclair. Regulation of human cytomegalovirus transcription in latency: Beyond the major immediate-early promoter. *Viruses*, 5(6):1395–1413, June 2013. ISSN 1999-4915. doi: 10.3390/v5061395. URL http://discovery.ucl.ac.uk/1406855/.

[157] David C. Bloom, Nicole V. Giordani, and Dacia L. Kwiatkowski. Epigenetic regulation of latent HSV-1 gene expression. *Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms*, 1799(34):246–256, March 2010. ISSN 1874-9399. doi: 10.1016/j.bbagrm.2009.12.001. URL http://www.sciencedirect.com/science/article/pii/S1874939909001667.

[158] Robert E. White, Ian J. Groves, Ernest Turro, Jade Yee, Elisabeth Kremmer, and Martin J. Allday. Extensive co-operation between the epstein-barr virus EBNA3 proteins in the manipulation of host gene expression and epigenetic

chromatin modification. *PLoS ONE*, 5(11):e13979, November 2010. doi: 10.1371/journal.pone.0013979. URL http://dx.doi.org/10.1371/journal.pone.0013979.

[159] Bjrn Grinde. Herpesviruses: latency and reactivation - viral strategies and host response. *Journal of oral microbiology*, 5, 2013. ISSN 2000-2297. doi: 10.3402/jom.v5i0.22766. PMID: 24167660 PMCID: PMC3809354.

[160] David C Bloom. HSV LAT and neuronal survival. *International reviews of immunology*, 23(1-2):187–198, April 2004. ISSN 0883-0185. PMID: 14690860.

[161] S C Verma, K Lan, and E Robertson. Structure and function of latency-associated nuclear antigen. *Current topics in microbiology and immunology*, 312:101–136, 2007. ISSN 0070-217X. PMID: 17089795 PMCID: PMC3142369.

[162] K. M. Kaye, K. M. Izumi, and E. Kieff. Epstein-barr virus latent membrane protein 1 is essential for b-lymphocyte growth transformation. *Proceedings of the National Academy of Sciences*, 90(19):9150–9154, October 1993. ISSN 0027-8424, 1091-6490. URL http://www.pnas.org/content/90/19/9150. PMID: 8415670.

[163] Jennifer S. Smith and N Jamie Robinson. Age-specific prevalence of infection with herpes simplex virus types 2 and 1: a global review. *J Infect Dis*, 186 Suppl 1:S3–28, Oct 2002. doi: 10.1086/343739. URL http://dx.doi.org/10.1086/343739.

[164] Sapna Modi, Livia Van, Aron Gewirtzman, Natalia Mendoza, Brenda Bartlett, Anne Marie Tremaine, and Stephen Tyring. Single-day treatment for orolabial and genital herpes: a brief review of pathogenesis and pharmacology. *Therapeutics and Clinical Risk Management*, 4(2):409–417, April 2008. ISSN 1176-6336. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2504076/. PMID: 18728852 PMCID: PMC2504076.

[165] M A O Lewis. Herpes simplex virus: an occupational hazard in dentistry. *International dental journal*, 54(2):103–111, April 2004. ISSN 0020-6539. PMID: 15119801.

[166] Asim V Farooq and Deepak Shukla. Herpes simplex epithelial and stromal keratitis: an epidemiologic update. *Survey of ophthalmology*, 57(5):448–462, September 2012. ISSN 1879-3304. doi: 10.1016/j.survophthal.2012.01.005. PMID: 22542912 PMCID: PMC3652623.

[167] Flore Rozenberg, Claire Deback, and Henri Agut. Herpes simplex encephalitis : from virus to therapy. *Infectious disorders drug targets*, 11(3):235–250, June 2011. ISSN 2212-3989. PMID: 21488834.

[168] Scott H. James, David W. Kimberlin, and Richard J. Whitley. Antiviral therapy for herpesvirus central nervous system infections: Neonatal herpes simplex virus infection, herpes simplex encephalitis, and congenital cytomegalovirus infection. *Antiviral Research*, 83(3):207–213, September 2009. ISSN 0166-3542. doi: 10.1016/j.antiviral.2009.04.010. URL http://www.sciencedirect.com/science/article/pii/S0166354209003209.

[169] Cariad M Evans, Goura Kudesia, and Mike McKendrick. Management of herpesvirus infections. *International journal of antimicrobial agents*, 42(2):119–128, August 2013. ISSN 1872-7913. doi: 10.1016/j.ijantimicag.2013.04.023. PMID: 23820015.

[170] David W. Kimberlin, Chin-Yu Lin, Richard F. Jacobs, Dwight A. Powell, Lawrence Corey, William C. Gruber, Mobeen Rathore, John S. Bradley, Pamela S. Diaz, Mary Kumar, Ann M. Arvin, Kathleen Gutierrez, Mark Shelton, Leonard B. Weiner, John W. Sleasman, Teresa Murgu?a de Sierra, Stephen Weller, Seng-Jaw Soong, Jan Kiell, Fred D. Lakeman, and Richard J. Whitley. Safety and efficacy of high-dose intravenous acyclovir in the management of neonatal herpes simplex virus infections. *Pediatrics*, 108(2):230–238, August 2001. ISSN 0031-4005, 1098-4275. doi: 10.1542/peds.108.2.230. URL http://pediatrics.aappublications.org/content/108/2/230. PMID: 11483782.

[171] Bishara J. Freij. Management of neonatal herpes simplex virus infections. *The Indian Journal of Pediatrics*, 71(10):921–926, October 2004. ISSN 0019-5456, 0973-7693. doi: 10.1007/BF02830837. URL http://link.springer.com/article/10.1007/BF02830837.

[172] S. I. M. Wolfert, E. P. de Jong, A. C. T. M. Vossen, J. Zwaveling, A. B. Te Pas, F. J. Walther, and E. Lopriore. Diagnostic and therapeutic management for suspected neonatal herpes simplex virus infection. *Jour-*

nal of Clinical Virology, 51(1):8–11, May 2011. ISSN 1386-6532. doi: 10.1016/j.jcv.2011.02.008. URL http://www.sciencedirect.com/science/article/pii/S138665321100076X.

[173] Lena J. Al-Dujaili, Patrick P. Clerkin, Christian Clement, Harris E. McFerrin, Partha S. Bhattacharjee, Emily D. Varnell, Herbert E. Kaufman, and James M. Hill. Ocular herpes simplex virus: how are latency, reactivation, recurrent disease and therapy interrelated? *Future Microbiol*, 6(8):877–907, Aug 2011. doi: 10.2217/fmb.11.73. URL http://dx.doi.org/10.2217/fmb.11.73.

[174] Jangu E. Banatvala. Herpes simplex encephalitis. *Lancet Infect Dis*, 11(2):80–81, Feb 2011. doi: 10.1016/S1473-3099(11)70012-3. URL http://dx.doi.org/10.1016/S1473-3099(11)70012-3.

[175] R J Whitley and B Roizman. Herpes simplex virus infections. *Lancet*, 357(9267):1513–1518, May 2001. ISSN 0140-6736. doi: 10.1016/S0140-6736(00)04638-9.

[176] Aziz Alami Chentoufi, Elizabeth Kritzer, David M. Yu, Anthony B. Nesburn, and Lbachir BenMohamed. Towards a rational design of an asymptomatic clinical herpes vaccine: The old, the new, and the unknown. *Clinical and Developmental Immunology*, 2012, 2012. ISSN 1740-2522. doi: 10.1155/2012/187585. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3324142/. PMID: 22548113 PMCID: PMC3324142.

[177] Jerry P. Weir. Regulation of herpes simplex virus gene expression. *Gene*, 271(2):117–130, June 2001. ISSN 0378-1119. doi: 10.1016/S0378-1119(01)00512-1. URL http://www.sciencedirect.com/science/article/pii/S0378111901005121.

[178] R.W. Honess and B. Roizman. Regulation of herpesvirus macromolecular synthesis. i. cascade regulation of the synthesis of three groups of viral proteins. *Journal of Virology*, 14(1):8–19, 1974. ISSN 0022-538X.

[179] F O Bastian, A S Rabson, C L Yee, and T S Tralka. Herpesvirus hominis: isolation from human trigeminal ganglion. *Science (New York, N.Y.)*, 178(4058):306–307, October 1972. ISSN 0036-8075. PMID: 4342752.

[180] C Jones. Alphaherpesvirus latency: its role in disease and survival of the virus in nature. *Advances in virus research*, 51:81–133, 1998. ISSN 0065-3527. PMID: 9891586.

[181] E K Wagner and D C Bloom. Experimental investigation of herpes simplex virus latency. *Clinical Microbiology Reviews*, 10(3):419–443, July 1997. ISSN 0893-8512. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC172928/. PMID: 9227860 PMCID: PMC172928.

[182] J M Lohr, J A Nelson, and M B Oldstone. Is herpes simplex virus associated with peptic ulcer disease? *Journal of Virology*, 64(5):2168–2174, May 1990. ISSN 0022-538X. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC249375/. PMID: 2157877 PMCID: PMC249375.

[183] R M Gesser and S C Koo. Latent herpes simplex virus type 1 gene expression in ganglia innervating the human gastrointestinal tract. *Journal of virology*, 71(5):4103–4106, May 1997. ISSN 0022-538X. PMID: 9094690 PMCID: PMC191565.

[184] Guey-Chuen Perng and Clinton Jones. Towards an understanding of the herpes simplex virus type 1 latency-reactivation cycle. *Interdisciplinary Perspectives on Infectious Diseases*, 2010, 2010. ISSN 1687-708X. doi: 10.1155/2010/262415. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2822239/. PMID: 20169002 PMCID: PMC2822239.

[185] Joanna Wysocka and Winship Herr. The herpes simplex virus VP16-induced complex: the makings of a regulatory switch. *Trends in Biochemical Sciences*, 28(6):294–304, June 2003. ISSN 0968-0004. doi: 10.1016/S0968-0004(03)00088-4. URL http://www.sciencedirect.com/science/article/pii/S0968000403000884.

[186] Michael Hagmann, Oleg Georgiev, Walter Schaffner, and Philippe Douville. Transcription factors interacting with herpes simplex virus gene promoters in sensory neurons. *Nucleic Acids Research*, 23(24):4978–4985, January 1995. ISSN 0305-1048, 1362-4962. doi: 10.1093/nar/23.24.4978. URL http://nar.oxfordjournals.org/content/23/24/4978.

[187] Robert T. Sarisky, Tammy T. Nguyen, Karen E. Duffy, Robert J. Wittrock, and Jeffry J. Leary. Difference in incidence of spontaneous mutations between herpes simplex virus types 1 and 2. *Antimicrobial Agents and*

Chemotherapy, 44(6):1524–1529, June 2000. ISSN 0066-4804, 1098-6596. doi: 10.1128/AAC.44.6.1524-1529.2000. URL http://aac.asm.org/content/44/6/1524. PMID: 10817703.

[188] D. R. Dubbs and Saul Kit. Mutant strains of herpes simplex deficient in thymidine kinase-inducing activity. *Virology*, 22(4):493–502, April 1964. ISSN 0042-6822. doi: 10.1016/0042-6822(64)90070-4. URL http://www.sciencedirect.com/science/article/pii/0042682264900704.

[189] Dianna E Wilkinson and Sandra K Weller. The role of DNA recombination in herpes simplex virus DNA replication. *IUBMB life*, 55(8):451–458, August 2003. ISSN 1521-6543. doi: 10.1080/15216540310001612237. PMID: 14609200.

[190] Jesper Melchjorsen. Sensing herpes: more than toll. *Rev Med Virol*, 22(2):106–121, Mar 2012. doi: 10.1002/rmv.716. URL http://dx.doi.org/10.1002/rmv.716.

[191] Jesper Melchjorsen, Johanna Rintahaka, Stine Sø by, Kristy a Horan, Alina Poltajainen, Lars Ø stergaard, Søren R Paludan, and Sampsa Matikainen. Early innate recognition of herpes simplex virus in human primary macrophages is mediated via the MDA5/MAVS-dependent and MDA5/MAVS/RNA polymerase III-independent pathways. *Journal of virology*, 84(21):11350–8, November 2010. ISSN 1098-5514. doi: 10.1128/JVI.01106-10. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2953193&tool=pmcentrez&rendertype=abstract.

[192] Y. Sokawa, T. Ando, and Y. Ishihara. Induction of 2',5'-oligoadenylate synthetase and interferon in mouse trigeminal ganglia infected with herpes simplex virus. *Infection and Immunity*, 28(3):719–723, June 1980. ISSN 0019-9567.

[193] Khaldun Al-khatib, Bryan R. G Williams, Robert H Silverman, William Halford, and Daniel J. J Carr. The murine double-stranded RNA-dependent protein kinase PKR and the murine 2,5-oligoadenylate synthetase-dependent RNase l are required for IFN--mediated resistance against herpes simplex virus type 1 in primary trigeminal ganglion culture. *Virology*, 313(1):126–135, August 2003. ISSN 0042-6822. doi: 10.1016/S0042-6822(03)00298-8. URL http://www.sciencedirect.com/science/article/pii/S0042682203002988.

[194] Daniel J. J. Carr, Khaldun Al-khatib, Cassandra M. James, and Robert Silverman. Interferon- suppresses herpes simplex virus type 1 replication in trigeminal ganglion cells through an RNase I-dependent pathway. *Journal of Neuroimmunology*, 141(12):40–46, August 2003. ISSN 0165-5728. doi: 10.1016/S0165-5728(03)00216-9. URL http://www.sciencedirect.com/science/article/pii/S0165572803002169.

[195] Bobbie Ann Austin, Cassandra James, Robert H. Silverman, and Daniel J. J. Carr. Critical role for the oligoadenylate Synthetase/RNase I pathway in response to IFN- during acute ocular herpes simplex virus type 1 infection. *The Journal of Immunology*, 175(2):1100–1106, July 2005. ISSN 0022-1767, 1550-6606. doi: 10.4049/jimmunol.175.2.1100. URL http://www.jimmunol.org/content/175/2/1100. PMID: 16002711.

[196] Ana Maria Low-Calle, Jeanette Prada-Arismendy, and Jaime E Castellanos. Study of interferon- antiviral activity against herpes simplex virus type 1 in neuron-enriched trigeminal ganglia cultures. *Virus research*, 180:49–58, February 2014. ISSN 1872-7492. doi: 10.1016/j.virusres.2013.12.022. PMID: 24374267.

[197] I Domke-Opitz, P Straub, and H Kirchner. Effect of interferon on replication of herpes simplex virus types 1 and 2 in human macrophages. *Journal of virology*, 60(1):37–42, October 1986. ISSN 0022-538X. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=253899&tool=pmcentrez&rendertype=abstract.

[198] I Domke, P Straub, H Jacobsen, H Kirchner, and A Panet. Inhibition of replication of herpes simplex virus in mouse macrophages by interferons. *The Journal of general virology*, 66 (Pt 10):2231–2236, October 1985. ISSN 0022-1317. PMID: 2413165.

[199] P Straub, I Domke, H Kirchner, H Jacobsen, and A Panet. Synthesis of herpes simplex virus proteins and nucleic acids in interferon-treated macrophages. *Virology*, 150(2):411–418, April 1986. ISSN 0042-6822. PMID: 2421481.

[200] F Oberman and A Panet. Inhibition of transcription of herpes simplex virus immediate early genes in interferon-treated human cells. *The Journal of general virology*, 69 (Pt 6):1167–1177, June 1988. ISSN 0022-1317. PMID: 2455018.

[201] I Gloger and A Panet. Synthesis of herpes simplex virus proteins in interferon-treated human cells. *The Journal of general virology*, 65 (Pt 6):1107–1111, June 1984. ISSN 0022-1317. PMID: 6327894.

[202] Gary D. Luker, Julie L. Prior, Jiling Song, Christina M. Pica, and David A. Leib. Bioluminescence imaging reveals systemic dissemination of herpes simplex virus type 1 in the absence of interferon receptors. *Journal of Virology*, 77 (20):11082–11093, October 2003. ISSN 0022-538X, 1098-5514. doi: 10.1128/JVI.77.20.11082-11093.2003. URL http://jvi.asm.org/content/77/20/11082. PMID: 14512556.

[203] Bo Cui and Daniel J. J. Carr. A plasmid construct encoding murine interferon beta antagonizes the replication of herpes simplex virus type i in vitro and in vivo. *Journal of Neuroimmunology*, 108(1):92–102, August 2000. ISSN 0165-5728. URL http://www.jni-journal.com/article/S0165-5728(00)00264-2/abstract.

[204] Khaldun Al-Khatib, Bryan R. G. Williams, Robert H. Silverman, William Halford, and Daniel J. J. Carr. Dichotomy between survival and lytic gene expression in RNase I- and PKR-deficient mice transduced with an adenoviral vector expressing murine IFN-beta following ocular HSV-1 infection. *Experimental Eye Research*, 80(2):167–173, February 2005. ISSN 0014-4835. doi: 10.1016/j.exer.2004.08.026.

[205] S. Noisakran, I. L. Campbell, and D. J. Carr. Ectopic expression of DNA encoding IFN-alpha 1 in the cornea protects mice from herpes simplex virus type 1-induced encephalitis. *Journal of Immunology (Baltimore, Md.: 1950)*, 162(7):4184–4190, April 1999. ISSN 0022-1767.

[206] Jia Guo, Weifeng Wang, Dongyang Yu, and Yuntao Wu. Spinoculation triggers dynamic actin and cofilin activity that facilitates hiv-1 infection of transformed and resting cd4 t cells. *J Virol*, 85(19):9824–9833, Oct 2011. doi: 10.1128/JVI.05170-11. URL http://dx.doi.org/10.1128/JVI.05170-11.

[207] Shen-Ying Zhang, Emmanuelle Jouanguy, Sophie Ugolini, Asma Smahi, Galle Elain, Pedro Romero, David Segal, Vanessa Sancho-Shimizu, Lazaro Lorenzo, Anne Puel, Capucine Picard, Ariane Chapgier, Sabine Plancoulaine, Matthias Titeux, Cline Cognet, Horst von Bernuth, Cheng-Lung Ku, Armanda Casrouge, Xin-Xin Zhang, Luis Barreiro, Joshua Leonard, Claire Hamilton, Pierre Lebon, Bndicte Hron, Louis Valle, Lluis Quintana-Murci, Alain Hovnanian, Flore Rozenberg, Eric Vivier, Frdric Geissmann, Marc Tardieu, Laurent Abel, and Jean-Laurent Casanova. TLR3 deficiency in patients with herpes simplex encephalitis. *Science (New York, N.Y.)*, 317(5844):1522–1527, September 2007. ISSN 1095-9203. doi: 10.1126/science.1139522.

[208] David A. Leib, Travis E. Harrison, Kathleen M. Laslo, Michael A. Machalek, Nathaniel J. Moorman, and Herbert W. Virgin. Interferons regulate the phenotype of wild-type and mutant herpes simplex viruses in vivo. *The Journal of Experimental Medicine*, 189(4):663 –672, February 1999. doi: 10.1084/jem.189.4.663.

[209] Ana Virginia Chee and Bernard Roizman. Herpes simplex virus 1 gene products occlude the interferon signaling pathway at multiple sites. *Journal of Virology*, 78(8):4185–4196, April 2004. ISSN 0022-538X, 1098-5514. doi: 10.1128/JVI.78.8.4185-4196.2004. URL http://jvi.asm.org/content/78/8/4185. PMID: 15047834.

[210] Gregory T Melroe, Neal A DeLuca, and David M Knipe. Herpes simplex virus 1 has multiple mechanisms for blocking virus-induced interferon production. *Journal of virology*, 78(16):8411–8420, August 2004. ISSN 0022-538X. doi: 10.1128/JVI.78.16.8411-8420.2004. PMID: 15280450 PMCID: PMC479070.

[211] Gregory T Melroe, Lindsey Silva, Priscilla A Schaffer, and David M Knipe. Recruitment of activated IRF-3 and CBP/p300 to herpes simplex virus ICP0 nuclear foci: Potential role in blocking IFN-beta induction. *Virology*, 360(2): 305–321, April 2007. ISSN 0042-6822. doi: 10.1016/j.virol.2006.10.028. PMID: 17126870 PMCID: PMC1976290.

[212] Shin-ichi Yokota, Noriko Yokosawa, Tamaki Okabayashi, Tatsuo Suzutani, Shunsuke Miura, Kowichi Jimbow, and Nobuhiro Fujii. Induction of suppressor of cytokine signaling-3 by herpes simplex virus type 1 contributes to inhibition of the interferon signaling pathway. *Journal of virology*, 78(12):6282–6286, June 2004. ISSN 0022-538X. doi: 10.1128/JVI.78.12.6282-6286.2004. PMID: 15163721 PMCID: PMC416529.

[213] Shin-ichi Yokota, Noriko Yokosawa, Tamaki Okabayashi, Tatsuo Suzutani, and Nobuhiro Fujii. Induction of suppressor of cytokine signaling-3 by herpes simplex virus type 1 confers efficient viral replication. *Virology*, 338(1): 173–181, July 2005. ISSN 0042-6822. doi: 10.1016/j.virol.2005.04.028. PMID: 15939448.

[214] Tomoki Todo. Active immunotherapy: oncolytic virus therapy using hsv-1. *Adv Exp Med Biol*, 746:178–186, 2012. doi: 10.1007/978-1-4614-3146-6_14. URL http://dx.doi.org/10.1007/978-1-4614-3146-6_14.

[215] Jacqueline Nuss Parker, David F Bauer, James J Cody, and James M Markert. Oncolytic viral therapy of malignant glioma. *Neurotherapeutics*, 6(3):558–569, Jul 2009. doi: 10.1016/j.nurt.2009.04.011. URL http://dx.doi.org/10.1016/j.nurt.2009.04.011.

[216] James M Markert, Jacqueline N Parker, Donald J Buchsbaum, William E Grizzle, G. Yancey Gillespie, and Richard J Whitley. Oncolytic hsv-1 for the treatment of brain tumours. *Herpes*, 13(3):66–71, Nov 2006.

[217] Gregory K Friedman, Joel Raborn, Virginia M Kelly, Kevin A Cassady, James M Markert, and G. Yancey Gillespie. Pediatric glioma stem cells: biologic strategies for oncolytic hsv virotherapy. *Front Oncol*, 3:28, 2013. doi: 10.3389/fonc.2013.00028. URL http://dx.doi.org/10.3389/fonc.2013.00028.

[218] Bernard Roizman, D. M. Knipe, and P. M. Howley. Herpes simplex viruses. In *Fields Virology (fifth ed.)*, pages 2501–2602. Lippincott, Williams, and Wilkins, Philadelphia, 2007.

[219] Israel Steiner, Peter GE Kennedy, and Andrew R Pachner. The neurotropic herpes viruses: herpes simplex and varicella-zoster. *The Lancet Neurology*, 6(11):1015–1028, November 2007. ISSN 1474-4422. doi: 10.1016/S1474-4422(07)70267-3. URL http://www.sciencedirect.com/science/article/pii/S1474442207702673.

[220] Isamu Mori. Herpes simplex virus us3 protein kinase regulates host responses and determines neurovirulence. *Microbiol Immunol*, 56(6):351–355, Jun 2012. doi: 10.1111/j.1348-0421.2012.00461.x. URL http://dx.doi.org/10.1111/j.1348-0421.2012.00461.x.

[221] Te Du, Guoying Zhou, and Bernard Roizman. Induction of apoptosis accelerates reactivation of latent HSV-1 in ganglionic organ cultures and replication in cell cultures. *Proceedings of the National Academy of Sciences of the United States of America*, 109(36):14616–14621, September 2012. ISSN 1091-6490. doi: 10.1073/pnas.1212661109. PMID: 22908263 PMCID: PMC3437834.

[222] Pradeep B J Reddy, Taylor H Schreiber, Naveen K Rajasagi, Amol Suryawanshi, Sachin Mulik, Tamara Veiga-Parga, Toshiro Niki, Mitsuomi Hirashima, Eckhard R Podack, and Barry T Rouse. Tnfrsf25 agonistic antibody and galectin-9 combination therapy controls herpes simplex virus-induced immunoinflammatory lesions. *J Virol*, 86 (19):10606–10620, Oct 2012. doi: 10.1128/JVI.01391-12. URL http://dx.doi.org/10.1128/JVI.01391-12.

[223] A. G. Colunga, J. M. Laing, and L. Aurelian. The hsv-2 mutant deltapk induces melanoma oncolysis through nonredundant death programs and associated with autophagy and pyroptosis proteins. *Gene Ther*, 17(3):315–327, Mar 2010. doi: 10.1038/gt.2009.126. URL http://dx.doi.org/10.1038/gt.2009.126.

[224] Piritta Peri, Kristiina Nuutila, Tytti Vuorinen, Pekka Saukko, and Veijo Hukkanen. Cathepsins are involved in virus-induced cell death in ICP4 and Us3 deletion mutant herpes simplex virus type 1-infected monocytic cells. *The Journal of general virology*, 92(Pt 1):173–80, January 2011. ISSN 1465-2099. doi: 10.1099/vir.0.025080-0. URL http://vir.sgmjournals.org/cgi/content/abstract/92/1/173.

[225] Ling Jin, Guey-Chuen Perng, Kevin R Mott, Nelson Osorio, Julia Naito, David J Brick, Dale Carpenter, Clinton Jones, and Steven L Wechsler. A herpes simplex virus type 1 mutant expressing a baculovirus inhibitor of apoptosis gene in place of latency-associated transcript has a wild-type reactivation phenotype in the mouse. *J Virol*, 79(19): 12286–12295, Oct 2005. doi: 10.1128/JVI.79.19.12286-12295.2005. URL http://dx.doi.org/10.1128/JVI.79.19.12286-12295.2005.

[226] Lei Jin, Paul M. Waterman, Karen R. Jonscher, Cindy M. Short, Nichole A. Reisdorph, and John C. Cambier. MPYS, a novel membrane tetraspanner, is associated with major histocompatibility complex class II and mediates transduction of apoptotic signals. *Mol. Cell. Biol.*, 28(16):5014–5026, August 2008. doi: 10.1128/MCB.00640-08.

[227] Kevin R Mott, Nelson Osorio, Ling Jin, David J Brick, Julie Naito, Jennifer Cooper, Gail Henderson, Melissa Inman, Clinton Jones, Steven L Wechsler, and Guey-Chuen Perng. The bovine herpesvirus-1 lr orf2 is critical for this gene's ability to restore the high wild-type reactivation phenotype to a herpes simplex virus-1 lat null mutant. *J Gen Virol*, 84(Pt 11):2975–2985, Nov 2003.

[228] Guey-Chuen Perng, Barak Maguen, Ling Jin, Kevin R Mott, Nelson Osorio, Susan M Slanina, Ada Yukht, Homayon Ghiasi, Anthony B Nesburn, Melissa Inman, Gail Henderson, Clinton Jones, and Steven L Wechsler. A gene capable of blocking apoptosis can substitute for the herpes simplex virus type 1 latency-associated transcript gene and restore wild-type reactivation levels. *J Virol*, 76(3):1224–1235, Feb 2002.

[229] Ghadah A. Karasneh and Deepak Shukla. Herpes simplex virus infects most cell types in vitro: clues to its success. *Virology Journal*, 8(1):481, October 2011. ISSN 1743-422X. doi: 10.1186/1743-422X-8-481. URL http://www.virologyj.com/content/8/1/481/abstract. PMID: 22029482.

[230] C a Daniels, E S Kleinerman, and R Snyderman. Abortive and productive infections of human mononuclear phagocytes by type I herpes simplex virus. *The American journal of pathology*, 91(1):119–36, April 1978. ISSN 0002-9440. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2018167&tool=pmcentrez&rendertype=abstract.

[231] R W Braun, H K Teute, H Kirchner, and K Munk. Replication of herpes simplex virus in human T lymphocytes: characterization of the viral target cell. *Journal of immunology (Baltimore, Md. : 1950)*, 132(2):914–9, February 1984. ISSN 0022-1767. URL http://www.ncbi.nlm.nih.gov/pubmed/6317753.

[232] I Albers, H Kirchner, and I Domke-Opitz. Resistance of human blood monocytes to infection with herpes simplex virus. *Virology*, 169(2):466–9, April 1989. ISSN 0042-6822. URL http://www.ncbi.nlm.nih.gov/pubmed/2539701.

[233] T Bruun, a K Kristoffersen, H Rollag, and M Degré. Interaction of herpes simplex virus with mononuclear phagocytes is dependent on the differentiation stage of the cells. *APMIS : acta pathologica, microbiologica, et immunologica Scandinavica*, 106(2):305–14, February 1998. ISSN 0903-4641. URL http://www.ncbi.nlm.nih.gov/pubmed/9531964.

[234] Alexandre Iannello, Olfa Debbeche, Raoudha El Arabi, Suzanne Samarani, David Hamel, Flore Rozenberg, Nikolaus Heveker, and Ali Ahmad. Herpes simplex virus type 1-induced FasL expression in human monocytic cells and its implications for cell death, viral replication, and immune evasion. *Viral immunology*, 24(1):11–26, February 2011. ISSN 1557-8976. doi: 10.1089/vim.2010.0083. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3117309&tool=pmcentrez&rendertype=abstract.

[235] S Hoves, H H Niller, S W Krause, R Straub, T Glück, J D Mountz, J Schölmerich, and M Fleck. Decreased T cell stimulatory capacity of monocyte-derived human macrophages following herpes simplex virus type 1 infection. *Scandinavian journal of immunology*, 54(1-2):93–9, 2001. ISSN 0300-9475. URL http://www.ncbi.nlm.nih.gov/pubmed/11439154.

[236] Richard T. Johnson. THE PATHOGENESIS OF HERPES VIRUS ENCEPHALITIS. *The Journal of Experimental Medicine*, 120(3):359–374, September 1964. ISSN 0022-1007. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2137762/.

[237] H Cheng, T M Tumpey, H F Staats, N van Rooijen, J E Oakes, and R N Lausch. Role of macrophages in restricting herpes simplex virus type 1 growth after ocular infection. *Investigative ophthalmology & visual science*, 41(6):1402–1409, May 2000. ISSN 0146-0404.

[238] D J Tenney and P S Morahan. Effects of differentiation of human macrophage-like U937 cells on intrinsic resistance to herpes simplex virus type 1. *Journal of immunology (Baltimore, Md. : 1950)*, 139(9):3076–83, November 1987. ISSN 0022-1767. URL http://www.ncbi.nlm.nih.gov/pubmed/2822803.

[239] Daniel J Tenney and Page S Morahan. Differentiation of the U937 macrophage cell line removes an early block of HSV-1 infection. *Viral Immunology*, 4(2):91–102, 1991.

[240] Stewart. Analysis of the Basis for Persistence of Herpes Simplex Virus Type 1 in Undifferentiated U937 Cells. *Viral immunology*, 5(3):173–184, 1992. URL http://www.liebertonline.com.libproxy.ucl.ac.uk/doi/abs/10.1089/vim.1992.5.173?prevSearch=allfield%253A%2528Stewart%2BAnaraki%2BLeary%2529&searchHistoryKey=.

[241] J a Lopez-Guerrero and M a Alonso. Nitric oxide production induced by herpes simplex virus type 1 does not alter the course of the infection in human monocytic cells. *The Journal of general virology*, 78 (Pt 8):1977–80, August 1997. ISSN 0022-1317. URL http://www.ncbi.nlm.nih.gov/pubmed/9266997.

[242] K Linnavuori and T Hovi. Restricted replication of herpes simplex virus in human monocyte cultures: role of interferon. *Virology*, 130(1):1–9, October 1983. ISSN 0042-6822. URL http://www.ncbi.nlm.nih.gov/pubmed/6195813.

[243] K Linnavuori and T Hovi. Herpes simplex virus as an inducer of interferon in human monocyte cultures. *Antiviral research*, 8(4):201–8, November 1987. ISSN 0166-3542. URL http://www.ncbi.nlm.nih.gov/pubmed/2833157.

[244] F. Capobianchi, M. R., Malavasi, P. , Di Marco, P. , Dianzani. Differences in the mechanism of induction of interferon-alpha by Herpes simplex virus and Herpes simplex virus-infected cells. *Archives of Virology*, 102:219–229, 1988.

[245] K H Linnavuori. History of recurrent mucocutaneous herpes correlates with relatively low interferon production by herpes simplex virus-exposed cultured monocytes. *Journal of medical virology*, 25(1):61–8, May 1988. ISSN 0146-6615. URL http://www.ncbi.nlm.nih.gov/pubmed/2842448.

[246] a Mastino, M T Sciortino, M a Medici, D Perri, M G Ammendolia, S Grelli, C Amici, a Pernice, and S Guglielmino. Herpes simplex virus 2 causes apoptotic infection in monocytoid cells. *Cell death and differentiation*, 4(7):629–38, October 1997. ISSN 1350-9047. doi: 10.1038/sj.cdd.4400289. URL http://www.ncbi.nlm.nih.gov/pubmed/14555977.

[247] Claudio Cermelli, Carlotta Francesca Orsi, Andrea Ardizzoni, Enrico Lugli, Valeria Cenacchi, Andrea Cossarizza, and Elisabetta Blasi. Herpes simplex virus type 1 dysregulates anti-fungal defenses preventing monocyte activation and downregulating toll-like receptor-2. *Microbiology and immunology*, 52(12):575–84, December 2008. ISSN 0385-5600. doi: 10.1111/j.1348-0421.2008.00074.x. URL http://www.ncbi.nlm.nih.gov/pubmed/19120971.

[248] Claudio Cermelli, Carlotta Francesca Orsi, Alessandro Cuoghi, Andrea Ardizzoni, Enrico Tagliafico, Rachele Neglia, Samuele Peppoloni, and Elisabetta Blasi. Gene expression profiling of monocytes displaying herpes simplex virus 1 induced dysregulation of antifungal defences. *Journal of medical microbiology*, 58(Pt 10):1283–90, October 2009. ISSN 1473-5644. doi: 10.1099/jmm.0.011023-0. URL http://www.ncbi.nlm.nih.gov/pubmed/19608693.

[249] Lene Malmgaard, Soren R. Paludan, Soren C. Mogensen, and Svend Ellermann-Eriksen. Herpes simplex virus type 2 induces secretion of IL-12 by macrophages through a mechanism involving NF-kappaB. *J Gen Virol*, 81(12): 3011–3020, December 2000.

[250] a L Cunningham, R R Turner, a C Miller, M F Para, and T C Merigan. Evolution of recurrent herpes simplex lesions. An immunohistologic study. *The Journal of clinical investigation*, 75(1):226–33, January 1985. ISSN 0021-9738. doi: 10.1172/JCI111678. URL http://www.ncbi.nlm.nih.gov/pubmed/18371288.

[251] Tao Peng, Jia Zhu, Alexis Klock, Khamsone Phasouk, Meei-Li Huang, David M. Koelle, Anna Wald, and Lawrence Corey. Evasion of the mucosal innate immune system by herpes simplex virus type 2. *J. Virol.*, 83(23):12559–12568, December 2009. doi: 10.1128/JVI.00939-09.

[252] Svend Ellermann-Eriksen. Macrophages and cytokines in the early defence against herpes simplex virus. *Virology journal*, 2:59, January 2005. ISSN 1743-422X. doi: 10.1186/1743-422X-2-59. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1215526&tool=pmcentrez&rendertype=abstract.

[253] Myron S. Cohen, Nick Hellmann, Jay A. Levy, Kevin DeCock, and Joep Lange. The spread, treatment, and prevention of HIV-1: evolution of a global pandemic. *Journal of Clinical Investigation*, 118(4):1244–1254, April 2008. ISSN 0021-9738. doi: 10.1172/JCI34706. URL http://www.jci.org/articles/view/34706.

[254] A. Engelman. Schematic overview of the hiv-1 replication cycle. *Nature Reviews Microbiology*, 10:279–290, 2012.

[255] L A Pereira, K Bentley, A Peeters, M J Churchill, and N J Deacon. A compilation of cellular transcription factor interactions with the HIV-1 LTR promoter. *Nucleic acids research*, 28(3):663–668, February 2000. ISSN 1362-4962. PMID: 10637316 PMCID: PMC102541.

[256] Robert J Danaher, Robert J Jacob, Marion R Steiner, Will R Allen, James M Hill, and Craig S Miller. Histone deacetylase inhibitors induce reactivation of herpes simplex virus type 1 in a latency-associated transcript-independent manner in neuronal cells. *Journal of neurovirology*, 11(3):306–317, July 2005. ISSN 1355-0284. doi: 10.1080/13550280590952817. PMID: 16036811 PMCID: PMC1361429.

[257] Mark Lucera, Carisa A. Tilton, Hongxia Mao, Curtis Dobrowolski, Caroline Tabler, Aiman A. Haqqani, Jonathan Karn, and John C. Tilton. The histone deacetylase inhibitor vorinostat (SAHA) increases the susceptibility of uninfected CD4+ t cells to HIV by increasing the kinetics and efficiency of post-entry viral events. *Journal of Virology*, pages JVI.00320–14, July 2014. ISSN 0022-538X, 1098-5514. doi: 10.1128/JVI.00320-14. URL `http://jvi.asm.org/content/early/2014/07/03/JVI.00320-14`.

[258] C Van Lint, S Emiliani, M Ott, and E Verdin. Transcriptional activation and chromatin remodeling of the HIV-1 promoter in response to histone acetylation. *The EMBO Journal*, 15(5):1112–1120, March 1996. ISSN 0261-4189. URL `http://www.ncbi.nlm.nih.gov/pmc/articles/PMC450009/`. PMID: 8605881 PMCID: PMC450009.

[259] Vincent Quivy, Emmanuelle Adam, Yves Collette, Dominique Demonte, Alain Chariot, Caroline Vanhulle, Ben Berkhout, Rmy Castellano, Yvan de Launoit, Arsne Burny, Jacques Piette, Vincent Bours, and Carine Van Lint. Synergistic activation of human immunodeficiency virus type 1 promoter activity by NF-B and inhibitors of deacetylases: Potential perspectives for the development of therapeutic strategies. *Journal of Virology*, 76(21): 11091–11103, November 2002. ISSN 0022-538X, 1098-5514. doi: 10.1128/JVI.76.21.11091-11103.2002. URL `http://jvi.asm.org/content/76/21/11091`. PMID: 12368351.

[260] R S Kornbluth, P S Oh, J R Munis, P H Cleveland, and D D Richman. Interferons and bacterial lipopolysaccharide protect macrophages from productive infection by human immunodeficiency virus in vitro. *The Journal of experimental medicine*, 169(3):1137–1151, March 1989. ISSN 0022-1007. PMID: 2466937 PMCID: PMC2189273.

[261] L Baca-Regen, N Heinzinger, M Stevenson, and H E Gendelman. Alpha interferon-induced antiretroviral activities: restriction of viral nucleic acid synthesis and progeny virion production in human immunodeficiency virus type 1-infected monocytes. *Journal of Virology*, 68(11):7559–7565, November 1994. ISSN 0022-538X.

[262] Kelly M Cheney and ?ine McKnight. Interferon-alpha mediates restriction of human immunodeficiency virus type-1 replication in primary human macrophages at an early stage of replication. *PloS one*, 5(10):e13521, 2010. ISSN 1932-6203. doi: 10.1371/journal.pone.0013521. PMID: 20975956 PMCID: PMC2958147.

[263] Mahdad Noursadeghi, Jhen Tsang, Robert F Miller, and David R Katz. Comment on "transcription factor foxo3a mediates apoptosis in hiv-1-infected macrophages". *J Immunol*, 180(12):7783; author reply 7783–7783; author reply 7784, Jun 2008.

[264] Edana Cassol, Massimo Alfano, Priscilla Biswas, and Guido Poli. Monocyte-derived macrophages and myeloid cell lines as targets of HIV-1 replication and persistence. *Journal of Leukocyte Biology*, 80(5):1018 –1030, November 2006. doi: 10.1189/jlb.0306150.

[265] Naimish R Patel, Jinping Zhu, Souvenir D Tachado, Jianmin Zhang, Zhi Wan, Jussi Saukkonen, and Henry Koziel. Hiv impairs tnf-alpha mediated macrophage apoptotic response to mycobacterium tuberculosis. *J Immunol*, 179 (10):6973–6980, Nov 2007.

[266] Simon Swingler, Angela M Mann, Jin Zhou, Catherine Swingler, and Mario Stevenson. Apoptotic killing of hiv-1-infected macrophages is subverted by the viral envelope glycoprotein. *PLoS Pathog*, 3(9):1281–1290, Sep 2007. doi: 10.1371/journal.ppat.0030134. URL `http://dx.doi.org/10.1371/journal.ppat.0030134`.

[267] Min Cui, Yunlong Huang, Yong Zhao, and Jialin Zheng. Transcription factor foxo3a mediates apoptosis in hiv-1-infected macrophages. *J Immunol*, 180(2):898–906, Jan 2008.

[268] Yunlong Huang, Nathan Erdmann, Hui Peng, Shelley Herek, John S Davis, Xu Luo, Tsuneya Ikezu, and Jialin Zheng. Trail-mediated apoptosis in hiv-1-infected macrophages is dependent on the inhibition of akt-1 phosphorylation. *J Immunol*, 177(4):2304–2313, Aug 2006.

[269] A. D. Badley, A. A. Pilon, A. Landay, and D. H. Lynch. Mechanisms of hiv-associated lymphocyte apoptosis. *Blood*, 96(9):2951–2964, Nov 2000.

[270] M. Kaul, G. A. Garden, and S. A. Lipton. Pathways to neuronal injury and apoptosis in hiv-associated dementia. *Nature*, 410(6831):988–994, Apr 2001. doi: 10.1038/35073667. URL http://dx.doi.org/10.1038/35073667.

[271] S. Polo, F. Veglia, M. S. Malnati, C. Gobbi, P. Farci, R. Raiteri, A. Sinicco, and P. Lusso. Longitudinal analysis of serum chemokine levels in the course of hiv-1 infection. *AIDS*, 13(4):447–454, Mar 1999.

[272] Martin D Hyrcza, Colin Kovacs, Mona Loutfy, Roberta Halpenny, Lawrence Heisler, Stuart Yang, Olivia Wilkins, Mario Ostrowski, and Sandy D Der. Distinct transcriptional profiles in ex vivo cd4+ and cd8+ t cells are established early in human immunodeficiency virus type 1 infection and are characterized by a chronic interferon response as well as extensive transcriptional changes in cd8+ t cells. *J Virol*, 81(7):3477–3486, Apr 2007. doi: 10.1128/JVI.01552-06. URL http://dx.doi.org/10.1128/JVI.01552-06.

[273] Michal Imbeault, Michel Ouellet, and Michel J Tremblay. Microarray study reveals that hiv-1 induces rapid type-i interferon-dependent p53 mrna up-regulation in human primary cd4+ t cells. *Retrovirology*, 6:5, 2009. doi: 10.1186/1742-4690-6-5. URL http://dx.doi.org/10.1186/1742-4690-6-5.

[274] Malavika S Giri, Michael Nebozhyn, Louise Showe, and Luis J Montaner. Microarray data on gene modulation by hiv-1 in immune cells: 2000-2006. *J Leukoc Biol*, 80(5):1031–1043, Nov 2006. doi: 10.1189/jlb.0306157. URL http://dx.doi.org/10.1189/jlb.0306157.

[275] Netanya G. Sandler, Steven E. Bosinger, Jacob D. Estes, Richard T. R. Zhu, Gregory K. Tharp, Eli Boritz, Doron Levin, Sathi Wijeyesinghe, Krystelle Nganou Makamdop, Gregory Q. del Prete, Brenna J. Hill, J. Katherina Timmer, Emma Reiss, Ganit Yarden, Samuel Darko, Eduardo Contijoch, John Paul Todd, Guido Silvestri, Martha Nason, Robert B. Norgren Jr, Brandon F. Keele, Srinivas Rao, Jerome A. Langer, Jeffrey D. Lifson, Gideon Schreiber, and Daniel C. Douek. Type i interferon responses in rhesus macaques prevent SIV infection and slow disease progression. *Nature*, 511(7511):601–605, July 2014. ISSN 0028-0836. doi: 10.1038/nature13554. URL http://www.nature.com.libproxy.ucl.ac.uk/nature/journal/v511/n7511/full/nature13554.html.

[276] Susan Moir, Angela Malaspina, Oxana K Pickeral, Eileen T Donoghue, Joshua Vasquez, Natalie J Miller, Surekha R Krishnan, Marie A Planta, John F Turney, J. Shawn Justement, Shyamasundaran Kottilil, Mark Dybul, JoAnn M Mican, Colin Kovacs, Tae-Wook Chun, Charles E Birse, and Anthony S Fauci. Decreased survival of b cells of hiv-viremic patients mediated by altered expression of receptors of the tnf superfamily. *J Exp Med*, 200(7):587–599, Oct 2004.

[277] Margalida Rotger, Kristen K Dang, Jacques Fellay, Erin L Heinzen, Sheng Feng, Patrick Descombes, Kevin V Shianna, Dongliang Ge, Huldrych F Gnthard, David B Goldstein, Amalio Telenti, Swiss HIV Cohort Study, and Center for HIV/AIDS Vaccine Immunology. Genome-wide mrna expression correlates of viral control in cd4+ t-cells from hiv-1-infected individuals. *PLoS Pathog*, 6(2):e1000781, Feb 2010. doi: 10.1371/journal.ppat.1000781. URL http://dx.doi.org/10.1371/journal.ppat.1000781.

[278] Steven E Bosinger, Karoline A Hosiawa, Mark J Cameron, Desmond Persad, Longsi Ran, Luoling Xu, Mohamed R Boulassel, Monique Parenteau, Jocelyn Fournier, Erling W Rud, and David J Kelvin. Gene expression profiling of host response in models of acute hiv infection. *J Immunol*, 173(11):6858–6863, Dec 2004.

[279] Jorge A. Tavel, Chiung-Yu Huang, Jean Shen, Julia A. Metcalf, Robin Dewar, Akram Shah, M. B. Vasudevachari, Dean A. Follmann, Betsey Herpin, Richard T. Davey, Michael A. Polis, Joseph Kovacs, Henry Masur, and H. Clifford Lane. Interferon-alpha produces significant decreases in HIV load. *Journal of Interferon & Cytokine Research: The Official Journal of the International Society for Interferon and Cytokine Research*, 30(7):461–464, July 2010. ISSN 1557-7465. doi: 10.1089/jir.2009.0090.

[280] X Wu, J A Conway, J Kim, and J C Kappes. Localization of the vpx packaging signal within the c terminus of the human immunodeficiency virus type 2 gag precursor protein. *Journal of virology*, 68(10):6161–6169, October 1994. ISSN 0022-538X. PMID: 8083957 PMCID: PMC237035.

[281] M A Accola, A A Bukovsky, M S Jones, and H G GÃ¶ttlinger. A conserved dileucine-containing motif in p6(gag) governs the particle association of vpx and vpr of simian immunodeficiency viruses SIV(mac) and SIV(agm). *Journal of Virology*, 73(12):9992–9999, December 1999. ISSN 0022-538X.

[282] L Selig, J C Pages, V Tanchou, S Prvral, C Berlioz-Torrent, L X Liu, L Erdtmann, J Darlix, R Benarous, and S Benichou. Interaction with the p6 domain of the gag precursor mediates incorporation into virions of vpr and vpx proteins from primate lentiviruses. *Journal of virology*, 73(1):592–600, January 1999. ISSN 0022-538X. PMID: 9847364 PMCID: PMC103865.

[283] X F Yu, Q C Yu, M Essex, and T H Lee. The vpx gene of simian immunodeficiency virus facilitates efficient viral replication in fresh lymphocytes and macrophage. *Journal of Virology*, 65(9):5088–5091, September 1991. ISSN 0022-538X.

[284] J S Gibbs, D A Regier, and R C Desrosiers. Construction and in vitro properties of HIV-1 mutants with deletions in "nonessential" genes. *AIDS Research and Human Retroviruses*, 10(4):343–350, April 1994. ISSN 0889-2229.

[285] I W Park and J Sodroski. Functional analysis of the vpx, vpr, and nef genes of simian immunodeficiency virus. *Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology: Official Publication of the International Retrovirology Association*, 8(4):335–344, April 1995. ISSN 1077-9450.

[286] T M Fletcher, B Brichacek, N Sharova, M A Newman, G Stivahtis, P M Sharp, M Emerman, B H Hahn, and M Stevenson. Nuclear import and cell cycle arrest functions of the HIV-1 vpr protein are encoded by two separate genes in HIV-2/SIV(SM). *The EMBO Journal*, 15(22):6155–6165, November 1996. ISSN 0261-4189.

[287] Fumiko Ueno, Hiroshi Shiota, Maki Miyaura, Akiko Yoshida, Akiko Sakurai, Junko Tatsuki, A Hajime Koyama, Hirofumi Akari, Akio Adachi, and Mikako Fujita. Vpx and vpr proteins of HIV-2 up-regulate the viral infectivity by a distinct mechanism in lymphocytic cells. *Microbes and infection / Institut Pasteur*, 5(5):387–395, April 2003. ISSN 1286-4579. PMID: 12737994.

[288] C Goujon, L Jarrosson-Wuillme, J Bernaud, D Rigal, J-L Darlix, and A Cimarelli. With a little help from a friend: increasing HIV transduction of monocyte-derived dendritic cells with virion-like particles of SIV(MAC). *Gene therapy*, 13(12):991–994, June 2006. ISSN 0969-7128. doi: 10.1038/sj.gt.3302753. PMID: 16525481.

[289] H A Pancio, N Vander Heyden, and L Ratner. The c-terminal proline-rich tail of human immunodeficiency virus type 2 vpx is necessary for nuclear localization of the viral preintegration complex in nondividing cells. *Journal of virology*, 74(13):6162–6167, July 2000. ISSN 0022-538X. PMID: 10846100 PMCID: PMC112115.

[290] S Mahalingam, B Van Tine, M L Santiago, F Gao, G M Shaw, and B H Hahn. Functional analysis of the simian immunodeficiency virus vpx protein: identification of packaging determinants and a novel nuclear targeting domain. *Journal of virology*, 75(1):362–374, January 2001. ISSN 0022-538X. doi: 10.1128/JVI.75.1.362-374.2001. PMID: 11119605 PMCID: PMC113929.

[291] C Depienne, P Roques, C Crminon, L Fritsch, R Casseron, D Dormont, C Dargemont, and S Benichou. Cellular distribution and karyophilic properties of matrix, integrase, and vpr proteins from the human and simian immunodeficiency viruses. *Experimental cell research*, 260(2):387–395, November 2000. ISSN 0014-4827. doi: 10.1006/excr.2000.5016. PMID: 11035935.

[292] Michael Belshan and Lee Ratner. Identification of the nuclear localization signal of human immunodeficiency virus type 2 vpx. *Virology*, 311(1):7–15, June 2003. ISSN 0042-6822. PMID: 12832198.

[293] Kasia Hrecka, Caili Hao, Magda Gierszewska, Selene K. Swanson, Malgorzata Kesik-Brodacka, Smita Srivastava, Laurence Florens, Michael P. Washburn, and Jacek Skowronski. Vpx relieves inhibition of hiv-1 infection of macrophages mediated by the samhd1 protein. *Nature*, 474(7353):658–661, Jun 2011. doi: 10.1038/nature10195. URL `http://dx.doi.org/10.1038/nature10195`.

[294] Nadine Laguette, Bijan Sobhian, Nicoletta Casartelli, Mathieu Ringeard, Christine Chable-Bessia, Emmanuel Sgral, Ahmad Yatim, Stphane Emiliani, Olivier Schwartz, and Monsef Benkirane. Samhd1 is the dendritic- and myeloid-cell-specific hiv-1 restriction factor counteracted by vpx. *Nature*, 474(7353):654–657, Jun 2011. doi: 10.1038/nature10117. URL `http://dx.doi.org/10.1038/nature10117`.

[295] Thomas Gramberg, Tanja Kahle, Nicolin Bloch, Sabine Wittmann, Erik Mllers, Waaqo Daddacha, Henning Hofmann, Baek Kim, Dirk Lindemann, and Nathaniel R Landau. Restriction of diverse retroviruses by SAMHD1. *Retrovirology*, 10:26, 2013. ISSN 1742-4690. doi: 10.1186/1742-4690-10-26. PMID: 23497255 PMCID: PMC3605129.

[296] Alexandre Sze, S Mehdi Belgnaoui, David Olagnier, Rongtuan Lin, John Hiscott, and Julien van Grevenynghe. Host restriction factor SAMHD1 limits human t cell leukemia virus type 1 infection of monocytes via STING-mediated apoptosis. *Cell host & microbe*, 14(4):422–434, October 2013. ISSN 1934-6069. doi: 10.1016/j.chom.2013.09.009. PMID: 24139400.

[297] Joseph A Hollenbaugh, Peter Gee, Jonathon Baker, Michele B Daly, Sarah M Amie, Jessica Tate, Natsumi Kasai, Yuka Kanemura, Dong-Hyun Kim, Brian M Ward, Yoshio Koyanagi, and Baek Kim. Host factor SAMHD1 restricts DNA viruses in non-dividing myeloid cells. *PLoS pathogens*, 9(6):e1003481, 2013. ISSN 1553-7374. doi: 10.1371/journal.ppat.1003481. PMID: 23825958 PMCID: PMC3694861.

[298] Eui Tae Kim, Tommy E White, Alberto Brandariz-Nez, Felipe Diaz-Griffero, and Matthew D Weitzman. SAMHD1 restricts herpes simplex virus 1 in macrophages by limiting DNA replication. *Journal of virology*, 87(23):12949–12956, December 2013. ISSN 1098-5514. doi: 10.1128/JVI.02291-13. PMID: 24067963 PMCID: PMC3838123.

[299] David C Goldstone, Valerie Ennis-Adeniran, Joseph J Hedden, Harriet C T Groom, Gillian I Rice, Evangelos Christodoulou, Philip A Walker, Geoff Kelly, Lesley F Haire, Melvyn W Yap, Luiz Pedro S de Carvalho, Jonathan P Stoye, Yanick J Crow, Ian A Taylor, and Michelle Webb. HIV-1 restriction factor SAMHD1 is a deoxynucleoside triphosphate triphosphohydrolase. *Nature*, 480(7377):379–382, December 2011. ISSN 1476-4687. doi: 10.1038/nature10623. PMID: 22056990.

[300] Rebecca D Powell, Paul J Holland, Thomas Hollis, and Fred W Perrino. Aicardi-goutieres syndrome gene and HIV-1 restriction factor SAMHD1 is a dGTP-regulated deoxynucleotide triphosphohydrolase. *The Journal of biological chemistry*, 286(51):43596–43600, December 2011. ISSN 1083-351X. doi: 10.1074/jbc.C111.317628. PMID: 22069334 PMCID: PMC3243528.

[301] Junpeng Yan, Sarabpreet Kaur, Maria DeLucia, Caili Hao, Jennifer Mehrens, Chuanping Wang, Marcin Golczak, Krzysztof Palczewski, Angela M Gronenborn, Jinwoo Ahn, and Jacek Skowronski. Tetramerization of SAMHD1 is required for biological activity and inhibition of HIV infection. *The Journal of biological chemistry*, 288(15):10406–10417, April 2013. ISSN 1083-351X. doi: 10.1074/jbc.M112.443796. PMID: 23426366 PMCID: PMC3624423.

[302] Hichem Lahouassa, Waaqo Daddacha, Henning Hofmann, Diana Ayinde, Eric C Logue, Loc Dragin, Nicolin Bloch, Claire Maudet, Matthieu Bertrand, Thomas Gramberg, Gianfranco Pancino, Stphane Priet, Bruno Canard, Nadine Laguette, Monsef Benkirane, Catherine Transy, Nathaniel R Landau, Baek Kim, and Florence Margottin-Goguet. SAMHD1 restricts the replication of human immunodeficiency virus type 1 by depleting the intracellular pool of deoxynucleoside triphosphates. *Nature immunology*, 13(3):223–228, March 2012. ISSN 1529-2916. doi: 10.1038/ni.2236. PMID: 22327569 PMCID: PMC3771401.

[303] Corine St Gelais, Suresh de Silva, Sarah M Amie, Christopher M Coleman, Heather Hoy, Joseph A Hollenbaugh, Baek Kim, and Li Wu. SAMHD1 restricts HIV-1 infection in dendritic cells (DCs) by dNTP depletion, but its expression in DCs and primary CD4+ t-lymphocytes cannot be upregulated by interferons. *Retrovirology*, 9:105, 2012. ISSN 1742-4690. doi: 10.1186/1742-4690-9-105. PMID: 23231760 PMCID: PMC3527137.

[304] Benjamin Descours, Alexandra Cribier, Christine Chable-Bessia, Diana Ayinde, Gillian Rice, Yanick Crow, Ahmad Yatim, Olivier Schwartz, Nadine Laguette, and Monsef Benkirane. SAMHD1 restricts HIV-1 reverse transcription in quiescent CD4(+) t-cells. *Retrovirology*, 9:87, 2012. ISSN 1742-4690. doi: 10.1186/1742-4690-9-87. PMID: 23092122 PMCID: PMC3494655.

[305] Alexandra Cribier, Benjamin Descours, Ana Luiza Chaves Valado, Nadine Laguette, and Monsef Benkirane. Phosphorylation of SAMHD1 by cyclin A2/CDK1 regulates its restriction activity toward HIV-1. *Cell reports*, 3(4): 1036–1043, April 2013. ISSN 2211-1247. doi: 10.1016/j.celrep.2013.03.017. PMID: 23602554.

[306] Tommy E White, Alberto Brandariz-Nuez, Jose Carlos Valle-Casuso, Sarah Amie, Laura Anh Nguyen, Baek Kim, Marina Tuzova, and Felipe Diaz-Griffero. The retroviral restriction ability of SAMHD1, but not its deoxynucleotide triphosphohydrolase activity, is regulated by phosphorylation. *Cell host & microbe*, 13(4):441–451, April 2013. ISSN 1934-6069. doi: 10.1016/j.chom.2013.03.005. PMID: 23601106 PMCID: PMC3864637.

[307] Tracy L Diamond, Mikhail Roshal, Varuni K Jamburuthugoda, Holly M Reynolds, Aaron R Merriam, Kwi Y

Lee, Mini Balakrishnan, Robert A Bambara, Vicente Planelles, Stephen Dewhurst, and Baek Kim. Macrophage tropism of HIV-1 depends on efficient cellular dNTP utilization by reverse transcriptase. *The Journal of biological chemistry*, 279(49):51545–51553, December 2004. ISSN 0021-9258. doi: 10.1074/jbc.M408573200. PMID: 15452123 PMCID: PMC1351161.

[308] Adriana Goncalves, Evren Karayel, Gillian I Rice, Keiryn L Bennett, Yanick J Crow, Giulio Superti-Furga, and Tilmann Brckstmmer. SAMHD1 is a nucleic-acid binding protein that is mislocalized due to aicardi-goutires syndrome-associated mutations. *Human mutation*, 33(7):1116–1122, July 2012. ISSN 1098-1004. doi: 10.1002/humu.22087. PMID: 22461318.

[309] Victoria Tngler, Wolfgang Staroske, Barbara Kind, Manuela Dobrick, Stefanie Kretschmer, Franziska Schmidt, Claudia Krug, Mike Lorenz, Osvaldo Chara, Petra Schwille, and Min Ae Lee-Kirsch. Single-stranded nucleic acids promote SAMHD1 complex formation. *Journal of molecular medicine (Berlin, Germany)*, 91(6):759–770, June 2013. ISSN 1432-1440. doi: 10.1007/s00109-013-0995-3. PMID: 23371319.

[310] Caroline Goujon, Vanessa Arfi, Thomas Pertel, Jeremy Luban, Julia Lienard, Dominique Rigal, Jean-Luc Darlix, and Andrea Cimarelli. Characterization of simian immunodeficiency virus SIVSM/human immunodeficiency virus type 2 vpx function in human myeloid cells. *Journal of Virology*, 82(24):12335–12345, December 2008. ISSN 1098-5514. doi: 10.1128/JVI.01181-08.

[311] Natalia Sharova, Yuanfei Wu, Xiaonan Zhu, Ruzena Stranska, Rajnish Kaushik, Mark Sharkey, and Mario Stevenson. Primate lentiviral vpx commandeers DDB1 to counteract a macrophage restriction. *PLoS Pathogens*, 4(5): e1000057, May 2008. ISSN 1553-7374. doi: 10.1371/journal.ppat.1000057.

[312] Smita Srivastava, Selene K Swanson, Nicolas Manel, Laurence Florens, Michael P Washburn, and Jacek Skowronski. Lentiviral vpx accessory factor targets VprBP/DCAF1 substrate adaptor for cullin 4 e3 ubiquitin ligase to enable macrophage infection. *PLoS Pathogens*, 4(5):e1000059, May 2008. ISSN 1553-7374. doi: 10.1371/journal.ppat.1000059.

[313] Anna Bergamaschi, Diana Ayinde, Annie David, Erwann Le Rouzic, Marina Morel, Gilles Collin, Diane Descamps, Florence Damond, Franoise Brun-Vezinet, Sebastien Nisole, Florence Margottin-Goguet, Gianfranco Pancino, and Catherine Transy. The human immunodeficiency virus type 2 vpx protein usurps the CUL4A-DDB1 DCAF1 ubiquitin ligase to overcome a postentry block in macrophage infection. *Journal of Virology*, 83(10):4854–4860, May 2009. ISSN 1098-5514. doi: 10.1128/JVI.00187-09.

[314] Thomas Gramberg, Nicole Sunseri, and Nathaniel R Landau. Evidence for an activation domain at the amino terminus of simian immunodeficiency virus vpx. *Journal of Virology*, 84(3):1387–1396, February 2010. ISSN 1098-5514. doi: 10.1128/JVI.01437-09.

[315] Thomas Pertel, Christian Reinhard, and Jeremy Luban. Vpx rescues HIV-1 transduction of dendritic cells from the antiviral state established by type 1 interferon. *Retrovirology*, 8:49, 2011. ISSN 1742-4690. doi: 10.1186/1742-4690-8-49. PMID: 21696578 PMCID: PMC3130655.

[316] Caroline Goujon, Torsten Schaller, Rui Pedro Galo, Sarah M Amie, Baek Kim, Kevin Olivieri, Stuart J D Neil, and Michael H Malim. Evidence for IFN-induced, SAMHD1-independent inhibitors of early HIV-1 infection. *Retrovirology*, 10:23, 2013. ISSN 1742-4690. doi: 10.1186/1742-4690-10-23. PMID: 23442224 PMCID: PMC3598776.

[317] Andr Berger, Carsten Mnk, Matthias Schweizer, Klaus Cichutek, Silke Schle, and Egbert Flory. Interaction of vpx and apolipoprotein b mRNA-editing catalytic polypeptide 3 family member a (APOBEC3A) correlates with efficient lentivirus infection of monocytes. *The Journal of biological chemistry*, 285(16):12248–12254, April 2010. ISSN 1083-351X. doi: 10.1074/jbc.M109.090977. PMID: 20178977 PMCID: PMC2852964.

[318] Fransje A Koning, Caroline Goujon, Hlne Bauby, and Michael H Malim. Target cell-mediated editing of HIV-1 cDNA by APOBEC3 proteins in human macrophages. *Journal of virology*, 85(24):13448–13452, December 2011. ISSN 1098-5514. doi: 10.1128/JVI.00775-11. PMID: 21957290 PMCID: PMC3233168.

[319] Michael H Malim and Paul D Bieniasz. HIV restriction factors and mechanisms of evasion. *Cold Spring Harbor*

perspectives in medicine, 2(5):a006940, May 2012. ISSN 2157-1422. doi: 10.1101/cshperspect.a006940. PMID: 22553496 PMCID: PMC3331687.

[320] Gang Peng, Ke Jian Lei, Wenwen Jin, Teresa Greenwell-Wild, and Sharon M Wahl. Induction of APOBEC3 family proteins, a defensive maneuver underlying interferon-induced anti-HIV-1 activity. *The Journal of Experimental Medicine*, 203(1):41–46, January 2006. ISSN 0022-1007. doi: 10.1084/jem.20051512.

[321] Eric W. Refsland, Mark D. Stenglein, Keisuke Shindo, John S. Albin, William L. Brown, and Reuben S. Harris. Quantitative profiling of the full APOBEC3 mRNA repertoire in lymphocytes and tissues: implications for HIV-1 restriction. *Nucleic Acids Research*, 38(13):4274–4284, July 2010. ISSN 1362-4962. doi: 10.1093/nar/gkq174.

[322] Gargi Dasgupta, Aziz A Chentoufi, Mina Kalantari, Payam Falatoonzadeh, Sookhee Chun, Chang Hyun Lim, Philip L Felgner, D Huw Davies, and Lbachir BenMohamed. Immunodominant "asymptomatic" herpes simplex virus 1 and 2 protein antigens identified by probing whole-ORFome microarrays with serum antibodies from seropositive asymptomatic versus symptomatic individuals. *Journal of virology*, 86(8):4358–4369, April 2012. ISSN 1098-5514. doi: 10.1128/JVI.07107-11. PMID: 22318137 PMCID: PMC3318627.

[323] Lara B Strick, Anna Wald, and Connie Celum. Management of herpes simplex virus type 2 infection in HIV type 1-infected persons. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America*, 43(3):347–356, August 2006. ISSN 1537-6591. doi: 10.1086/505496. PMID: 16804851.

[324] Anna M. Foss, Peter T. Vickerman, Zaid Chalabi, Philippe Mayaud, Michel Alary, and Charlotte H. Watts. Dynamic modeling of herpes simplex virus type-2 (HSV-2) transmission: Issues in structural uncertainty. *Bulletin of Mathematical Biology*, 71(3):720–749, April 2009. ISSN 0092-8240, 1522-9602. doi: 10.1007/s11538-008-9379-1. URL http://link.springer.com/article/10.1007/s11538-008-9379-1.

[325] E.E. Freeman, H.A. Weiss, J.R. Glynn, P.L. Cross, J.A. Whitworth, and R.J. Hayes. Herpes simplex virus 2 infection increases HIV acquisition in men and women: Systematic review and meta-analysis of longitudinal studies. *AIDS*, 20(1):73–83, 2006. ISSN 0269-9370.

[326] A. Wald and K. Link. Risk of human immunodeficiency virus infection in herpes simplex virus type 2-seropositive persons: A meta-analysis. *Journal of Infectious Diseases*, 185(1):45–52, 2002. ISSN 0022-1899. doi: 10.1086/338231.

[327] R.H. Gray, X. Li, M.J. Wawer, D. Serwadda, N.K. Sewankambo, F. Wabwire-Mangen, T. Lutalo, N. Kiwanuka, G. Kigozi, F. Nalugoda, M.P. Meehan, M. Robb, and T.C. Quinn. Determinants of HIV-1 load in subjects with early and later HIV infections, in a general-population cohort of rakai, uganda. *Journal of Infectious Diseases*, 189 (7):1209–1215, 2004. ISSN 0022-1899. doi: 10.1086/382750.

[328] T. Schacker, J. Zeh, H. Hu, M. Shaughnessy, and L. Corey. Changes in plasma human immunodeficiency virus type 1 RNA associated with herpes simplex virus reactivation and suppression. *Journal of Infectious Diseases*, 186 (12):1718–1725, 2002. ISSN 0022-1899. doi: 10.1086/345771.

[329] S.M. Blower and L. Ma. Calculating the contribution of herpes simplex virus type 2 epidemics to increasing HIV incidence: Treatment implications. *Clinical Infectious Diseases*, 39(SUPPL. 5):S240–S247, 2004. ISSN 1058-4838. doi: 10.1086/422361.

[330] R.G. White, E.E. Freeman, K.K. Orroth, R. Bakker, H.A. Weiss, N. O'Farrell, A. Buv, R.J. Hayes, and J.R. Glynn. Population-level effect of HSV-2 therapy on the incidence of HIV in sub-saharan africa. *Sexually Transmitted Infections*, 84(SUPPL. 2):ii12–ii18, 2008. ISSN 1368-4973. doi: 10.1136/sti.2008.029918.

[331] Laith J. Abu-Raddad, Amalia S. Magaret, Connie Celum, Anna Wald, Jr. Longini, Ira M., Steven G. Self, and Lawrence Corey. Genital herpes has played a more important role than any other sexually transmitted infection in driving HIV prevalence in africa. *PLoS ONE*, 3(5):e2230, May 2008. doi: 10.1371/journal.pone.0002230. URL http://dx.plos.org/10.1371/journal.pone.0002230.

[332] Anthony N Muiru, Brandon L Guthrie, Rose Bosire, Michele Merkel, Amy Y Liu, Robert Y Choi, Barbara Lohman-Payne, Ann Gatuguta, Romel D Mackelprang, James N Kiarie, and Carey Farquhar. Incident HSV-2 infections are

common among HIV-1-discordant couples. *The Journal of infectious diseases*, 208(7):1093–1101, October 2013. ISSN 1537-6613. doi: 10.1093/infdis/jit303. PMID: 23840044 PMCID: PMC3762386.

[333] Jim Todd, Gabriele Riedner, Leonard Maboko, Michael Hoelscher, Helen A Weiss, Eligius Lyamuya, David Mabey, Mary Rusizoka, Laurent Belec, and Richard Hayes. Effect of genital herpes on cervicovaginal HIV shedding in women co-infected with HIV AND HSV-2 in tanzania. *PloS one*, 8(3):e59037, 2013. ISSN 1932-6203. doi: 10.1371/journal.pone.0059037. PMID: 23516595 PMCID: PMC3596319.

[334] Jairam R Lingappa and Connie Celum. Clinical and therapeutic issues for herpes simplex virus-2 and HIV co-infection. *Drugs*, 67(2):155–174, 2007. ISSN 0012-6667. PMID: 17284082.

[335] Connie L Celum. The interaction between herpes simplex virus and human immunodeficiency virus. *Herpes: the journal of the IHMF*, 11 Suppl 1:36A–45A, April 2004. ISSN 0969-7667. PMID: 15115628.

[336] Julie E Horbul, Stephen C Schmechel, Barrie R L Miller, Stephen A Rice, and Peter J Southern. Herpes simplex virus-induced epithelial damage and susceptibility to human immunodeficiency virus type 1 infection in human cervical organ culture. *PLoS One*, 6(7):e22638, 2011. doi: 10.1371/journal.pone.0022638. URL `http://dx.doi.org/10.1371/journal.pone.0022638`.

[337] Tamarind M Keating, Ann E Kurth, Anna Wald, Erin M Kahle, Elizabeth A Barash, and Susan E Buskin. Clinical burden of herpes simplex virus disease in people with human immunodeficiency virus. *Sex Transm Dis*, 39(5):372–376, May 2012. doi: 10.1097/OLQ.0b013e318244ac4c. URL `http://dx.doi.org/10.1097/OLQ.0b013e318244ac4c`.

[338] Ruanne V Barnabas and Connie Celum. Infectious co-factors in HIV-1 transmission herpes simplex virus type-2 and HIV-1: new insights and interventions. *Current HIV research*, 10(3):228–237, April 2012. ISSN 1873-4251. PMID: 22384842 PMCID: PMC3563330.

[339] Jared M Baeten, Lara B Strick, Aldo Lucchetti, William L H Whittington, Jorge Sanchez, Robert W Coombs, Amalia Magaret, Anna Wald, Lawrence Corey, and Connie Celum. Herpes simplex virus (HSV)-suppressive therapy decreases plasma and genital HIV-1 levels in HSV-2/HIV-1 coinfected women: a randomized, placebo-controlled, cross-over trial. *The Journal of infectious diseases*, 198(12):1804–1808, December 2008. ISSN 0022-1899. doi: 10.1086/593214. PMID: 18928378 PMCID: PMC2665183.

[340] Philippe Van de Perre, Michel Segondy, Vincent Foulongne, Abdoulaye Ouedraogo, Issouf Konate, Jean-Marie Huraux, Philippe Mayaud, and Nicolas Nagot. Herpes simplex virus and HIV-1: deciphering viral synergy. *The Lancet infectious diseases*, 8(8):490–497, August 2008. ISSN 1473-3099. doi: 10.1016/S1473-3099(08)70181-6. PMID: 18652995.

[341] Nicolas Nagot, Abdoulaye Oudraogo, Vincent Foulongne, Issouf Konat, Helen A Weiss, Laurence Vergne, Marie-Christine Defer, Didier Djagbar, Anselme Sanon, Jean-Baptiste Andonaba, Pierre Becquart, Michel Segondy, Roselyne Vallo, Adrien Sawadogo, Philippe Van de Perre, Philippe Mayaud, and ANRS 1285 Study Group. Reduction of HIV-1 RNA levels with therapy to suppress herpes simplex virus. *The New England journal of medicine*, 356(8):790–799, February 2007. ISSN 1533-4406. doi: 10.1056/NEJMoa062607. PMID: 17314338.

[342] P Mayaud, N Nagot, I Konat, A Ouedraogo, H A Weiss, V Foulongne, M-C Defer, A Sawadogo, M Segondy, P Van de Perre, and ANRS 1285 Study Group. Effect of HIV-1 and antiretroviral therapy on herpes simplex virus type 2: a prospective study in african women. *Sexually transmitted infections*, 84(5):332–337, October 2008. ISSN 1472-3263. doi: 10.1136/sti.2008.030692. PMID: 18596069.

[343] Pierre-Alain Rubbo, Edouard Tuaillon, Nicolas Nagot, Aziz Alami Chentoufi, Karine Bollor, Jacques Reynes, Jean-Pierre Vendrell, Lbachir BenMohamed, and Philippe Van De Perre. HIV-1 infection impairs HSV-specific CD4(+) and CD8(+) t-cell response by reducing th1 cytokines and CCR5 ligand secretion. *Journal of acquired immune deficiency syndromes (1999)*, 58(1):9–17, September 2011. ISSN 1944-7884. doi: 10.1097/QAI.0b013e318224d0ad. PMID: 21646911.

[344] Zhilan Feng, Zhipeng Qiu, Zi Sang, Christina Lorenzo, and John Glasser. Modeling the synergy between HSV-2

and HIV and potential impact of HSV-2 therapy. *Mathematical biosciences*, 245(2):171–187, October 2013. ISSN 1879-3134. doi: 10.1016/j.mbs.2013.07.003. PMID: 23850537.

[345] T Schacker, A J Ryncarz, J Goddard, K Diem, M Shaughnessy, and L Corey. Frequent recovery of HIV-1 from genital herpes simplex virus lesions in HIV-1-infected men. *JAMA: the journal of the American Medical Association*, 280(1):61–66, July 1998. ISSN 0098-7484. PMID: 9660365.

[346] Jia Zhu, Florian Hladik, Amanda Woodward, Alexis Klock, Tao Peng, Christine Johnston, Michael Remington, Amalia Magaret, David M Koelle, Anna Wald, and Lawrence Corey. Persistence of HIV-1 receptor-positive cells after HSV-2 reactivation is a potential mechanism for increased HIV-1 acquisition. *Nature medicine*, 15(8):886–892, August 2009. ISSN 1546-170X. doi: 10.1038/nm.2006. PMID: 19648930 PMCID: PMC2723183.

[347] Anuradha Rebbapragada, Charles Wachihi, Christopher Pettengell, Sherzana Sunderji, Sanja Huibner, Walter Jaoko, Blake Ball, Keith Fowke, Tony Mazzulli, Francis A Plummer, and Rupert Kaul. Negative mucosal synergy between herpes simplex type 2 and HIV in the female genital tract. *AIDS (London, England)*, 21(5):589–598, March 2007. ISSN 0269-9370. doi: 10.1097/QAD.0b013e328012b896. PMID: 17314521.

[348] Marein A W P de Jong, Lot de Witte, Maureen E Taylor, and Teunis B H Geijtenbeek. Herpes simplex virus type 2 enhances HIV-1 susceptibility by affecting langerhans cell function. *Journal of immunology (Baltimore, Md.: 1950)*, 185(3):1633–1641, August 2010. ISSN 1550-6606. doi: 10.4049/jimmunol.0904137. PMID: 20592277.

[349] C M Posavad, D M Koelle, M F Shaughnessy, and L Corey. Severe genital herpes infections in HIV-infected individuals with impaired herpes simplex virus-specific CD8+ cytotoxic t lymphocyte responses. *Proceedings of the National Academy of Sciences of the United States of America*, 94(19):10289–10294, September 1997. ISSN 0027-8424. PMID: 9294203 PMCID: PMC23355.

[350] Lawrence Corey, Anna Wald, Connie L Celum, and Thomas C Quinn. The effects of herpes simplex virus-2 on HIV-1 acquisition and transmission: a review of two overlapping epidemics. *Journal of acquired immune deficiency syndromes (1999)*, 35(5):435–445, April 2004. ISSN 1525-4135. PMID: 15021308.

[351] T Schacker, H L Hu, D M Koelle, J Zeh, R Saltzman, R Boon, M Shaughnessy, G Barnum, and L Corey. Famciclovir for the suppression of symptomatic and asymptomatic herpes simplex virus reactivation in HIV-infected persons. a double-blind, placebo-controlled trial. *Annals of internal medicine*, 128(1):21–28, January 1998. ISSN 0003-4819. PMID: 9424977.

[352] Kristine E Johnson, Andrew D Redd, Thomas C Quinn, Aleisha N Collinson-Streng, Toby Cornish, Xiangrong Kong, Rajni Sharma, Aaron A R Tobian, Benjamin Tsai, Mark E Sherman, Godfrey Kigozi, David Serwadda, Maria J Wawer, and Ronald H Gray. Effects of HIV-1 and herpes simplex virus type 2 infection on lymphocyte and dendritic cell density in adult foreskins from rakai, uganda. *The Journal of infectious diseases*, 203(5):602–609, March 2011. ISSN 1537-6613. doi: 10.1093/infdis/jiq091. PMID: 21220779 PMCID: PMC3071278.

[353] Rumi Minami, Masahiro Yamamoto, Soichiro Takahama, Hitoshi Ando, Tomoya Miyamura, and Eiichi Suematsu. Human herpesvirus 8 DNA load in the leukocytes correlates with the platelet counts in HIV type 1-infected individuals. *AIDS research and human retroviruses*, 25(1):1–8, January 2009. ISSN 1931-8405. doi: 10.1089/aid.2007.0260. PMID: 19182916.

[354] Antonino Carbone, Ethel Cesarman, Michele Spina, Annunziata Gloghini, and Thomas F Schulz. HIV-associated lymphomas and gamma-herpesviruses. *Blood*, 113(6):1213–1224, February 2009. ISSN 1528-0020. doi: 10.1182/blood-2008-09-180315. PMID: 18955561.

[355] Elizabeth Griffin, Elizabeth Krantz, Stacy Selke, Meei-Li Huang, and Anna Wald. Oral mucosal reactivation rates of herpesviruses among HIV-1 seropositive persons. *Journal of medical virology*, 80(7):1153–1159, July 2008. ISSN 0146-6615. doi: 10.1002/jmv.21214. PMID: 18461621.

[356] Jay A Levy. HIV pathogenesis: 25 years of progress and persistent challenges. *AIDS (London, England)*, 23(2):147–160, January 2009. ISSN 1473-5571. doi: 10.1097/QAD.0b013e3283217f9f. PMID: 19098484.

[357] A S Fauci, G Pantaleo, S Stanley, and D Weissman. Immunopathogenic mechanisms of HIV infection. *Annals of internal medicine*, 124(7):654–663, April 1996. ISSN 0003-4819. PMID: 8607594.

[358] Irna Sufiawati and Sharof M Tugizov. HIV-associated disruption of tight and adherens junctions of oral epithelial cells facilitates HSV-1 infection and spread. *PloS one*, 9(2):e88803, 2014. ISSN 1932-6203. doi: 10.1371/journal.pone.0088803. PMID: 24586397 PMCID: PMC3931628.

[359] Ling Bai, Zhenping Zhang, Hui Zhang, Xiumei Li, Qiurong Yu, Haotian Lin, and Wenhui Yang. HIV-1 tat protein alter the tight junction integrity and function of retinal pigment epithelium: an in vitro study. *BMC infectious diseases*, 8:77, 2008. ISSN 1471-2334. doi: 10.1186/1471-2334-8-77. PMID: 18538010 PMCID: PMC2430207.

[360] Georgette D Kanmogne, Charles Primeaux, and Paula Grammas. HIV-1 gp120 proteins alter tight junction protein expression and brain endothelial cell permeability: implications for the pathogenesis of HIV-associated dementia. *Journal of neuropathology and experimental neurology*, 64(6):498–505, June 2005. ISSN 0022-3069. PMID: 15977641.

[361] Shinichi Nakamuta, Hiroshi Endo, Youichiro Higashi, Aoi Kousaka, Hiroshi Yamada, Mihiro Yano, and Hiroshi Kido. Human immunodeficiency virus type 1 gp120-mediated disruption of tight junction proteins by induction of proteasome-mediated degradation of zonula occludens-1 and -2 in human brain microvascular endothelial cells. *Journal of NeuroVirology*, 14(3):186–195, May 2008. ISSN 1355-0284, 1538-2443. doi: 10.1080/13550280801993630. URL http://link.springer.com/article/10.1080/13550280801993630.

[362] Andrew H Karaba, Sarah J Kopp, and Richard Longnecker. Herpesvirus entry mediator and nectin-1 mediate herpes simplex virus 1 infection of the murine cornea. *Journal of virology*, 85(19):10041–10047, October 2011. ISSN 1098-5514. doi: 10.1128/JVI.05445-11. PMID: 21795335 PMCID: PMC3196397.

[363] Sarah A Connolly, Daniel J Landsburg, Andrea Carfi, J Charles Whitbeck, Yi Zuo, Don C Wiley, Gary H Cohen, and Roselyn J Eisenberg. Potential nectin-1 binding site on herpes simplex virus glycoprotein d. *Journal of virology*, 79(2):1282–1295, January 2005. ISSN 0022-538X. doi: 10.1128/JVI.79.2.1282-1295.2005. PMID: 15613355 PMCID: PMC538551.

[364] F Cocchi, M Lopez, P Dubreuil, G Campadelli Fiume, and L Menotti. Chimeric nectin1-poliovirus receptor molecules identify a nectin1 region functional in herpes simplex virus entry. *Journal of virology*, 75(17):7987–7994, September 2001. ISSN 0022-538X. PMID: 11483743 PMCID: PMC115042.

[365] Miri Yoon and Patricia G Spear. Disruption of adherens junctions liberates nectin-1 to serve as receptor for herpes simplex virus and pseudorabies virus entry. *Journal of virology*, 76(14):7203–7208, July 2002. ISSN 0022-538X. PMID: 12072519 PMCID: PMC136315.

[366] Meghna Ramaswamy and Anna Maria Geretti. Interactions and management issues in hsv and hiv coinfection. *Expert Rev Anti Infect Ther*, 5(2):231–243, Apr 2007. doi: 10.1586/14787210.5.2.231. URL http://dx.doi.org/10.1586/14787210.5.2.231.

[367] S L Schafer, J Vlach, and P M Pitha. Cooperation between herpes simplex virus type 1-encoded ICP0 and tat to support transcription of human immunodeficiency virus type 1 long terminal repeat in vivo can occur in the absence of the TAR binding site. *Journal of virology*, 70(10):6937–6946, October 1996. ISSN 0022-538X. PMID: 8794337 PMCID: PMC190743.

[368] D M Margolis, A B Rabson, S E Straus, and J M Ostrove. Transactivation of the HIV-1 LTR by HSV-1 immediate-early genes. *Virology*, 186(2):788–791, February 1992. ISSN 0042-6822. PMID: 1310199.

[369] Patty W Wright, Craig J Hoesley, Kathleen E Squires, Angela Croom-Rivers, Heidi L Weiss, and Jr Gnann, John W. A prospective study of genital herpes simplex virus type 2 infection in human immunodeficiency virus type 1 (HIV-1)-seropositive women: correlations with CD4 cell count and plasma HIV-1 RNA level. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America*, 36(2):207–211, January 2003. ISSN 1537-6591. doi: 10.1086/345440. PMID: 12522754.

[370] L S Kucera, E Leake, N Iyer, D Raben, and Q N Myrvik. Human immunodeficiency virus type 1 (HIV-1) and herpes simplex virus type 2 (HSV-2) can coinfect and simultaneously replicate in the same human CD4+ cell: effect of coinfection on infectious HSV-2 and HIV-1 replication. *AIDS research and human retroviruses*, 6(5):641–647, May 1990. ISSN 0889-2229. PMID: 1972888.

[371] E K Bagdades, D Pillay, S B Squire, C O'Neil, M A Johnson, and P D Griffiths. Relationship between herpes simplex virus ulceration and CD4+ cell counts in patients with HIV infection. *AIDS (London, England)*, 6(11): 1317–1320, November 1992. ISSN 0269-9370. PMID: 1361745.

[372] Santhi Gorantla, Kathlyn Santos, Vakara Meyer, Stephen Dewhurst, William J Bowers, Howard J Federoff, Howard E Gendelman, and Larisa Poluektova. Human dendritic cells transduced with herpes simplex virus amplicons encoding human immunodeficiency virus type 1 (HIV-1) gp120 elicit adaptive immune responses from human cells engrafted into NOD/SCID mice and confer partial protection against HIV-1 challenge. *Journal of virology*, 79(4):2124–2132, February 2005. ISSN 0022-538X. doi: 10.1128/JVI.79.4.2124-2132.2005. PMID: 15681415 PMCID: PMC546587.

[373] Elena Sartori, Arianna Calistri, Cristiano Salata, Claudia Del Vecchio, Giorgio Pal, and Cristina Parolin. Herpes simplex virus type 2 infection increases human immunodeficiency virus type 1 entry into human primary macrophages. *Virology Journal*, 8(1):166, April 2011. ISSN 1743-422X. doi: 10.1186/1743-422X-8-166. URL http://www.virologyj.com/content/8/1/166/abstract. PMID: 21486479.

[374] M Moriuchi, H Moriuchi, R Williams, and S E Straus. Herpes simplex virus infection induces replication of human immunodeficiency virus type 1. *Virology*, 278(2):534–540, December 2000. ISSN 0042-6822. doi: 10.1006/viro.2000.0667. PMID: 11118375.

[375] Taneth Yamsuwan, Chintana Chirathaworn, Pokrath Hansasuta, and Parvapan Bhattarakosol. HIV-1 replication in HIV-infected individuals is significantly reduced when peripheral blood mononuclear cells are superinfected with HSV-1. *TheScientificWorldJournal*, 2012:102843, 2012. ISSN 1537-744X. doi: 10.1100/2012/102843. PMID: 22973164 PMCID: PMC3438744.

[376] Mahdad Noursadeghi, Jhen Tsang, Robert F. Miller, Sarah Straschewski, Paul Kellam, Benjamin M. Chain, and David R. Katz. Genome-wide innate immune responses in hiv-1-infected macrophages are preserved despite attenuation of the nf-kappa b activation pathway. *J Immunol*, 182(1):319–328, Jan 2009.

[377] J. M. Tsang. *Innate Immune Responses in HIV-1 Infected Macrophages*. Doctoral, UCL (University College London), January 2014. URL http://discovery.ucl.ac.uk/1417140/.

[378] S Al-Nasiry, N Geusens, M Hanssens, C Luyten, and R Pijnenborg. The use of alamar blue assay for quantitative analysis of viability, migration and invasion of choriocarcinoma cells. *Human reproduction (Oxford, England)*, 22 (5):1304–1309, May 2007. ISSN 0268-1161. doi: 10.1093/humrep/dem011. PMID: 17307808.

[379] John Quinn. Roll your own FCS files part 2. URL http://flowjo.typepad.com/the_daily_dongle/2010/12/roll-your-own-fcs-files-part-2.html.

[380] T. J. Hill, H. J. Field, and W. A. Blyth. Acute and recurrent infection with herpes simplex virus in the mouse: a model for studying latency and recurrent disease. *Journal of General Virology*, 28(3):341–353, September 1975. ISSN 0022-1317, 1465-2099. doi: 10.1099/0022-1317-28-3-341. URL http://vir.sgmjournals.org/content/28/3/341. PMID: 170376.

[381] Ann L. Cornish, Rachael Keating, Konstantinos Kyparissoudis, Mark J. Smyth, Francis R. Carbone, and Dale I. Godfrey. NKT cells are not critical for HSV-1 disease resolution. *Immunology and Cell Biology*, 84(1):13–19, December 2005. ISSN 0818-9641. doi: 10.1111/j.1440-1711.2005.01396.x. URL http://www.nature.com/icb/journal/v84/n1/full/icb20063a.html.

[382] Ekaterina E Heldwein, Huan Lou, Florent C Bender, Gary H Cohen, Roselyn J Eisenberg, and Stephen C Harrison. Crystal structure of glycoprotein b from herpes simplex virus 1. *Science (New York, N.Y.)*, 313(5784):217–220, July 2006. ISSN 1095-9203. doi: 10.1126/science.1126548. PMID: 16840698.

[383] Stphane Roche, Stphane Bressanelli, Flix A Rey, and Yves Gaudin. Crystal structure of the low-pH form of the vesicular stomatitis virus glycoprotein g. *Science (New York, N.Y.)*, 313(5784):187–191, July 2006. ISSN 1095-9203. doi: 10.1126/science.1127683. PMID: 16840692.

[384] Jan Kadlec, Silvia Loureiro, Nicola G A Abrescia, David I Stuart, and Ian M Jones. The postfusion structure of baculovirus gp64 supports a unified view of viral fusion machines. *Nature structural & molecular biology*, 15(10): 1024–1030, October 2008. ISSN 1545-9985. doi: 10.1038/nsmb.1484. PMID: 18776902.

[385] Annarita Falanga, Rossella Tarallo, Giuseppe Vitiello, Mariateresa Vitiello, Emiliana Perillo, Marco Cantisani, Gerardino D'Errico, Massimiliano Galdiero, and Stefania Galdiero. Biophysical characterization and membrane interaction of the two fusion loops of glycoprotein b from herpes simplex type i virus. *PLoS ONE*, 7(2):e32186, February 2012. doi: 10.1371/journal.pone.0032186. URL http://dx.doi.org/10.1371/journal.pone.0032186.

[386] Adi Reske, Gabriele Pollara, Claude Krummenacher, Benjamin M. Chain, and David R. Katz. Understanding HSV-1 entry glycoproteins. *Reviews in Medical Virology*, 17(3):205–215, May 2007. ISSN 1099-1654. doi: 10.1002/rmv.531. URL http://onlinelibrary.wiley.com/doi/10.1002/rmv.531/abstract.

[387] Sebastian Temme, Anna M Eis-Hbinger, Alexander D McLellan, and Norbert Koch. The herpes simplex virus-1 encoded glycoprotein b diverts HLA-DR into the exosome pathway. *Journal of immunology (Baltimore, Md.: 1950)*, 184(1):236–243, January 2010. ISSN 1550-6606. doi: 10.4049/jimmunol.0902192. PMID: 19949097.

[388] Ping Rao, Hong Thanh Pham, Arpita Kulkarni, Yang Yang, Xueqiao Liu, David M Knipe, Peter Cresswell, and Weiming Yuan. Herpes simplex virus 1 glycoprotein b and US3 collaborate to inhibit CD1d antigen presentation and NKT cell function. *Journal of virology*, 85(16):8093–8104, August 2011. ISSN 1098-5514. doi: 10.1128/JVI.02689-10. PMID: 21653669 PMCID: PMC3147970.

[389] Miles C Smith, Chris Boutell, and David J Davido. HSV-1 ICP0: paving the way for viral replication. *Future virology*, 6(4):421–429, April 2011. ISSN 1746-0794. doi: 10.2217/fvl.11.24. PMID: 21765858 PMCID: PMC3133933.

[390] W P Halford, C D Kemp, J A Isler, D J Davido, and P A Schaffer. ICP0, ICP4, or VP16 expressed from adenovirus vectors induces reactivation of latent herpes simplex virus type 1 in primary cultures of latently infected trigeminal ganglion cells. *Journal of virology*, 75(13):6143–6153, July 2001. ISSN 0022-538X. doi: 10.1128/JVI.75.13.6143-6153.2001. PMID: 11390616 PMCID: PMC114330.

[391] W P Halford and P A Schaffer. ICP0 is required for efficient reactivation of herpes simplex virus type 1 from neuronal latency. *Journal of virology*, 75(7):3240–3249, April 2001. ISSN 0022-538X. doi: 10.1128/JVI.75.7.3240-3249.2001. PMID: 11238850 PMCID: PMC114117.

[392] R. A. Harris, R. D. Everett, X. X. Zhu, S. Silverstein, and C. M. Preston. Herpes simplex virus type 1 immediate-early protein vmw110 reactivates latent herpes simplex virus type 2 in an in vitro latency system. *Journal of Virology*, 63(8):3513–3515, August 1989. ISSN 0022-538X, 1098-5514. URL http://jvi.asm.org/content/63/8/3513. PMID: 2545921.

[393] W Cai, T L Astor, L M Liptak, C Cho, D M Coen, and P A Schaffer. The herpes simplex virus type 1 regulatory protein ICP0 enhances virus replication during acute infection and reactivation from latency. *Journal of Virology*, 67(12):7501–7512, December 1993. ISSN 0022-538X. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC238216/.

[394] S Plaeger-Marshall. Replication of Herpes Simplex Virus in Blood Monocytes and Placental macrophages from Human Neonates. *Proceedings of the Society for Experimental Biology and Medicine*, 26(2):135–139, 1989.

[395] Jesper Melchjorsen, Jukka Siren, Ilkka Julkunen, Soren R. Paludan, and Sampsa Matikainen. Induction of cytokine expression by herpes simplex virus in human monocyte-derived macrophages and dendritic cells is dependent on virus replication and is counteracted by ICP27 targeting NF-kappaB and IRF-3. *J Gen Virol*, 87(5):1099–1108, May 2006. doi: 10.1099/vir.0.81541-0.

[396] B J Montagnon, B Fanget, and A J Nicolas. The large-scale cultivation of VERO cells in micro-carrier culture for virus vaccine production. preliminary results for killed poliovirus vaccine. *Developments in biological standardization*, 47:55–64, 1981. ISSN 0301-5149. PMID: 6785126.

[397] Jan Desmyter, Joseph L. Melnick, and William E. Rawls. Defectiveness of interferon production and of rubella virus interference in a line of african green monkey kidney cells (vero). *Journal of Virology*, 2(10):955–961, October 1968. ISSN 0022-538X. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC375423/. PMID: 4302013 PMCID: PMC375423.

[398] James Brian Wechuck. PRODUCTION AND PURIFICATION OF HSV-1 VECTORS AND ITS USE FOR GENE TRANSFER TO HUMAN CD34? CELLS, March 2002. URL http://d-scholarship.pitt.edu/6368/.

[399] Peter A Gillis, Laura H Okagaki, and Stephen A Rice. Herpes simplex virus type 1 icp27 induces p38 mitogen-activated protein kinase signaling and apoptosis in hela cells. *J Virol*, 83(4):1767–1777, Feb 2009. doi: 10.1128/JVI.01944-08. URL http://dx.doi.org/10.1128/JVI.01944-08.

[400] M. Aubert, J. O'Toole, and J. A. Blaho. Induction and prevention of apoptosis in human hep-2 cells by herpes simplex virus type 1. *J Virol*, 73(12):10359–10370, Dec 1999.

[401] Danna Hargett, Stephen Rice, and Steven L. Bachenheimer. Herpes simplex virus type 1 ICP27-Dependent activation of NF-?B. *Journal of Virology*, 80(21):10565–10578, November 2006. ISSN 0022-538X. doi: 10.1128/JVI.01119-06. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1641752/.

[402] D WuDunn and P G Spear. Initial interaction of herpes simplex virus with cells is binding to heparan sulfate. *Journal of Virology*, 63(1):52–58, January 1989. ISSN 0022-538X. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC247656/.

[403] B C Herold, S I Gerber, B J Belval, A M Siston, and N Shulman. Differences in the susceptibility of herpes simplex virus types 1 and 2 to modified heparin compounds suggest serotype differences in viral entry. *Journal of Virology*, 70 (6):3461–3469, June 1996. ISSN 0022-538X. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC190219/.

[404] Ben Davidson, Hiep Phuc Dong, Aasmund Berner, and Bjrn Risberg. The diagnostic and research applications of flow cytometry in cytopathology. *Diagn Cytopathol*, 40(6):525–535, Jun 2012. doi: 10.1002/dc.22809. URL http://dx.doi.org/10.1002/dc.22809.

[405] Patricia G Spear. Herpes simplex virus: receptors and ligands for cell entry. *Cellular microbiology*, 6(5):401–410, May 2004. ISSN 1462-5814. doi: 10.1111/j.1462-5822.2004.00389.x.

[406] Juho J Miettinen, Sampsa Matikainen, and Tuula A Nyman. Global secretome characterization of herpes simplex virus 1-infected human primary macrophages. *J Virol*, 86(23):12770–12778, Dec 2012. doi: 10.1128/JVI.01545-12. URL http://dx.doi.org/10.1128/JVI.01545-12.

[407] Petr Broz, Jakob von Moltke, Jonathan W Jones, Russell E Vance, and Denise M Monack. Differential requirement for caspase-1 autoproteolysis in pathogen-induced cell death and cytokine processing. *Cell Host Microbe*, 8(6): 471–483, Dec 2010. doi: 10.1016/j.chom.2010.11.007. URL http://dx.doi.org/10.1016/j.chom.2010.11.007.

[408] Laurie A. Dempsey. Interferon-induced necroptosis. *Nature Immunology*, 14(9):892–892, September 2013. ISSN 1529-2908. doi: 10.1038/ni.2700. URL http://www.nature.com/ni/journal/v14/n9/full/ni.2700.html.

[409] Jason W. Upton, William J. Kaiser, and Edward S. Mocarski. Dai/zbp1/dlm-1 complexes with rip3 to mediate virus-induced programmed necrosis that is targeted by murine cytomegalovirus vira. *Cell Host Microbe*, 11(3):290–297, Mar 2012. doi: 10.1016/j.chom.2012.01.016. URL http://dx.doi.org/10.1016/j.chom.2012.01.016.

[410] Jian Zou, Taro Kawai, Tetsuo Tsuchida, Tatsuya Kozaki, Hiroki Tanaka, Kyung-Sue Shin, Himanshu Kumar, and Shizuo Akira. Poly IC triggers a cathepsin d- and IPS-1-dependent pathway to enhance cytokine production and mediate dendritic cell necroptosis. *Immunity*, 38(4):717–728, April 2013. ISSN 1097-4180. doi: 10.1016/j.immuni.2012.12.007. PMID: 23601685.

[411] Sudan He, Lai Wang, Lin Miao, Tao Wang, Fenghe Du, Liping Zhao, and Xiaodong Wang. Receptor interacting protein kinase-3 determines cellular necrotic response to TNF-. *Cell*, 137(6):1100–1111, June 2009. ISSN 0092-8674. doi: 10.1016/j.cell.2009.05.021. URL http://www.cell.com/abstract/S0092-8674(09)00578-9.

[412] Duan-Wu Zhang, Jing Shao, Juan Lin, Na Zhang, Bao-Ju Lu, Sheng-Cai Lin, Meng-Qiu Dong, and Jiahuai Han. RIP3, an energy metabolism regulator that switches TNF-induced cell death from apoptosis to necrosis. *Science (New York, N.Y.)*, 325(5938):332–336, July 2009. ISSN 1095-9203. doi: 10.1126/science.1172308. PMID: 19498109.

[413] Piritta Peri, Riikka K Mattila, Helena Kantola, Eeva Broberg, Heidi S Karttunen, Matti Waris, Tytti Vuorinen, and Veijo Hukkanen. Herpes simplex virus type 1 Us3 gene deletion influences toll-like receptor responses in cultured monocytic cells. *Virology journal*, 5:140, January 2008. ISSN 1743-422X. doi:

10.1186/1743-422X-5-140. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2605447&tool=pmcentrez&rendertype=abstract.

[414] Shinichi Esaki, Fumi Goshima, Sachiyo Katsumi, Daisuke Watanabe, Noriyuki Ozaki, Shingo Murakami, and Yukihiro Nishiyama. Apoptosis induction after herpes simplex virus infection differs according to cell type in vivo. *Arch Virol*, 155(8):1235–1245, Aug 2010. doi: 10.1007/s00705-010-0712-2. URL http://dx.doi.org/10.1007/s00705-010-0712-2.

[415] J. Munger and B. Roizman. The us3 protein kinase of herpes simplex virus 1 mediates the posttranslational modification of bad and prevents bad-induced programmed cell death in the absence of other viral proteins. *Proc Natl Acad Sci U S A*, 98(18):10410–10415, Aug 2001. doi: 10.1073/pnas.181344498. URL http://dx.doi.org/10.1073/pnas.181344498.

[416] Paul D Ogg, Peter J McDonell, Brent J Ryckman, C. Michael Knudson, and Richard J Roller. The hsv-1 us3 protein kinase is sufficient to block apoptosis induced by overexpression of a variety of bcl-2 family members. *Virology*, 319(2):212–224, Feb 2004. doi: 10.1016/j.virol.2003.10.019. URL http://dx.doi.org/10.1016/j.virol.2003.10.019.

[417] C Lopez and G Dudas. Replication of herpes simplex virus type 1 in macrophages from resistant and susceptible mice. *Infection and immunity*, 23(2):432–7, February 1979. ISSN 0019-9567. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=414183&tool=pmcentrez&rendertype=abstract.

[418] K Leary, J R Connor, and P S Morahan. Comparison of herpes simplex virus type 1 DNA replication and virus production in murine bone marrow-derived and resident peritoneal macrophages. *The Journal of general virology*, 66 (Pt 5):1123–9, May 1985. ISSN 0022-1317. URL http://www.ncbi.nlm.nih.gov/pubmed/2987398.

[419] J G Stevens and M L Cook. Restriction of herpes simplex virus by macrophages. An analysis of the cell-virus interaction. *The Journal of experimental medicine*, 133(1):19–38, January 1971. ISSN 0022-1007. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2138889&tool=pmcentrez&rendertype=abstract.

[420] Sarmiento M. Mouse Macrophage Restriction of Herpes Simplex Virus Replication. *Veterinary Medicine*, 141(8):2740–2748, 1988. URL http://www.ncbi.nlm.nih.gov/pubmed?term=Intrinsicresistancetoviralinfection%3Amousemacrophagerestrictionofherpessimplexvirusreplication.J.

[421] M F Sit, D J Tenney, J L Rothstein, and P S Morahan. Effect of macrophage activation on resistance of mouse peritoneal macrophages to infection with herpes simplex virus types 1 and 2. *The Journal of general virology*, 69 (Pt 8)(1988):1999–2010, August 1988. ISSN 0022-1317. URL http://www.ncbi.nlm.nih.gov/pubmed/2841412.

[422] Kevin R Mott, David Underhill, Steven L Wechsler, Terrence Town, and Homayon Ghiasi. A role for the JAK-STAT1 pathway in blocking replication of HSV-1 in dendritic cells and macrophages. *Virology journal*, 6:56, January 2009. ISSN 1743-422X. doi: 10.1186/1743-422X-6-56. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2686698&tool=pmcentrez&rendertype=abstract.

[423] Yijie Ma and Bin He. Recognition of herpes simplex viruses: toll-like receptors and beyond. *Journal of molecular biology*, 426(6):1133–1147, March 2014. ISSN 1089-8638. doi: 10.1016/j.jmb.2013.11.012.

[424] Jie Zhang, Kezhen Wang, Shuai Wang, and Chunfu Zheng. Herpes simplex virus 1 e3 ubiquitin ligase ICP0 protein inhibits tumor necrosis factor alpha-induced NF-B activation by interacting with p65/RelA and p50/NF-B1. *Journal of virology*, 87(23):12935–12948, December 2013. ISSN 1098-5514. doi: 10.1128/JVI.01952-13. PMID: 24067962 PMCID: PMC3838126.

[425] Sandrine Daubeuf, Divyendu Singh, Yaohong Tan, Hongiu Liu, Howard J Federoff, William J Bowers, and Khaled Tolba. HSV ICP0 recruits USP7 to modulate TLR-mediated innate response. *Blood*, 113(14):3264–3275, April 2009. ISSN 1528-0020. doi: 10.1182/blood-2008-07-168203. PMID: 18952891 PMCID: PMC3401030.

[426] Tatiana Gianni, Valerio Leoni, and Gabriella Campadelli-Fiume. Type i interferon and NF-B activation elicited by herpes simplex virus gH/gL via v3 integrin in epithelial and neuronal cell lines. *Journal of virology*, 87(24):13911–13916, December 2013. ISSN 1098-5514. doi: 10.1128/JVI.01894-13. PMID: 24109241 PMCID: PMC3838217.

[427] Junji Xing, Liwen Ni, Shuai Wang, Kezhen Wang, Rongtuan Lin, and Chunfu Zheng. Herpes simplex virus 1-encoded tegument protein VP16 abrogates the production of beta interferon (IFN) by inhibiting NF-b activation and blocking IFN regulatory factor 3 to recruit its coactivator CBP. *Journal of Virology*, 87(17):9788–9801, September 2013. ISSN 1098-5514. doi: 10.1128/JVI.01440-13.

[428] Mingsheng Cai, Meili Li, Kezhen Wang, Shuai Wang, Qiong Lu, Jinghua Yan, Karen L Mossman, Rongtuan Lin, and Chunfu Zheng. The herpes simplex virus 1-encoded envelope glycoprotein b activates NF-B through the toll-like receptor 2 and MyD88/TRAF6-dependent signaling pathway. *PloS one*, 8(1):e54586, 2013. ISSN 1932-6203. doi: 10.1371/journal.pone.0054586. PMID: 23382920 PMCID: PMC3557241.

[429] Gilad Doitsh, Nicole L. K. Galloway, Xin Geng, Zhiyuan Yang, Kathryn M. Monroe, Orlando Zepeda, Peter W. Hunt, Hiroyu Hatano, Stefanie Sowinski, Isa Muoz-Arias, and Warner C. Greene. Cell death by pyroptosis drives CD4 t-cell depletion in HIV-1 infection. *Nature*, 505(7484):509–514, January 2014. ISSN 0028-0836. doi: 10.1038/nature12940. URL http://www.nature.com/nature/journal/v505/n7484/full/nature12940.html.

[430] Ting Pan, Shuangxin Wu, Xin He, Haihua Luo, Yijun Zhang, Miaomiao Fan, Guannan Geng, Vivian Clarke Ruiz, Jim Zhang, Lisa Mills, Chuan Bai, and Hui Zhang. Necroptosis takes place in human immunodeficiency virus type-1 (HIV-1)-infected CD4+ t lymphocytes. *PloS One*, 9(4):e93944, 2014. ISSN 1932-6203. doi: 10.1371/journal.pone.0093944.

[431] Reuben S. Harris, Judd F. Hultquist, and David T. Evans. The restriction factors of human immunodeficiency virus. *Journal of Biological Chemistry*, 287(49):40875–40883, November 2012. ISSN 0021-9258, 1083-351X. doi: 10.1074/jbc.R112.416925. URL http://www.jbc.org/content/287/49/40875.

[432] Ritu Goila-Gaur, Mohammad A. Khan, Eri Miyagi, Sandra Kao, Sandrine Opi, Hiroaki Takeuchi, and Klaus Strebel. HIV-1 vif promotes the formation of high molecular mass APOBEC3g complexes. *Virology*, 372(1):136–146, March 2008. ISSN 0042-6822. doi: 10.1016/j.virol.2007.10.017.

[433] Kunitoshi Chiba, Junichi Yamamoto, Yuki Yamaguchi, and Hiroshi Handa. Promoter-proximal pausing and its release: molecular mechanisms and physiological functions. *Experimental Cell Research*, 316(17):2723–2730, October 2010. ISSN 1090-2422. doi: 10.1016/j.yexcr.2010.05.036.

[434] A. Marcello, M. Zopp, and M. Giacca. Multiple modes of transcriptional regulation by the HIV-1 tat transactivator. *IUBMB life*, 51(3):175–181, March 2001. ISSN 1521-6543. doi: 10.1080/152165401753544241.

[435] Stefanie Jager, Peter Cimermancic, Natali Gulbahce, Jeffrey R. Johnson, Kathryn E. McGovern, Starlynn C. Clarke, Michael Shales, Gaelle Mercenne, Lars Pache, Kathy Li, Hilda Hernandez, Gwendolyn M. Jang, Shoshannah L. Roth, Eyal Akiva, John Marlett, Melanie Stephens, Ivn D'Orso, Jason Fernandes, Marie Fahey, Cathal Mahon, Anthony J. O'Donoghue, Aleksandar Todorovic, John H. Morris, David A. Maltby, Tom Alber, Gerard Cagney, Frederic D. Bushman, John A. Young, Sumit K. Chanda, Wesley I. Sundquist, Tanja Kortemme, Ryan D. Hernandez, Charles S. Craik, Alma Burlingame, Andrej Sali, Alan D. Frankel, and Nevan J. Krogan. Global landscape of HIV-human protein complexes. *Nature*, 481(7381):365–370, January 2012. ISSN 1476-4687. doi: 10.1038/nature10719.

[436] Melanie Ott, Matthias Geyer, and Qiang Zhou. The control of HIV transcription: keeping RNA polymerase II on track. *Cell Host & Microbe*, 10(5):426–435, November 2011. ISSN 1934-6069. doi: 10.1016/j.chom.2011.11.002.

[437] David M. Lukac, Jessica R. Kirshner, and Don Ganem. Transcriptional activation by the product of open reading frame 50 of kaposis sarcoma-associated herpesvirus is required for lytic viral reactivation in b cells. *Journal of Virology*, 73(11):9348–9361, November 1999. ISSN 0022-538X, 1098-5514. URL http://jvi.asm.org/content/73/11/9348. PMID: 10516043.

[438] Elisabetta Caselli, Paola Menegazzi, Arianna Bracci, Monica Galvan, Enzo Cassai, and Dario Di Luca. Human herpesvirus-8 (kaposis sarcoma-associated herpesvirus) ORF50 interacts synergistically with the tat gene product in transactivating the human immunodeficiency virus type 1 LTR. *Journal of General Virology*, 82(8):1965–1970, August 2001. ISSN 0022-1317, 1465-2099. URL http://vir.sgmjournals.org/content/82/8/1965. PMID: 11458004.

[439] Elisabetta Caselli, Monica Galvan, Enzo Cassai, Arnaldo Caruso, Laura Sighinolfi, and Dario Di Luca. Human herpesvirus 8 enhances human immunodeficiency virus replication in acutely infected cells and induces reactivation in latently infected cells. *Blood*, 106(8):2790–2797, October 2005. ISSN 0006-4971, 1528-0020. doi: 10.1182/blood-2005-04-1390. URL http://bloodjournal.hematologylibrary.org/content/106/8/2790. PMID: 15976177.

[440] F. Bachelerie, J. Alcami, F. Arenzana-Seisdedos, and J.-L. Virelizier. HIV enhancer activity perpetuated by NF-b induction on infection of monocytes. *Nature*, 350(6320):709–712, April 1991. doi: 10.1038/350709a0. URL http://www.nature.com.libproxy.ucl.ac.uk/nature/journal/v350/n6320/abs/350709a0.html.

[441] P D de Maisieres, L Baudoux-Tebache, M P Merville, B Rentier, V Bours, and J Piette. Activation of the human immunodeficiency virus long terminal repeat by varicella-zoster virus IE4 protein requires nuclear factor-kappaB and involves both the amino-terminal and the carboxyl-terminal cysteine-rich region. *The Journal of biological chemistry*, 273(22):13636–13644, May 1998. ISSN 0021-9258. PMID: 9593702.

[442] L J Strobl, H Hfelmayr, C Stein, G Marschall, M Brielmeier, G Laux, G W Bornkamm, and U Zimber-Strobl. Both epstein-barr viral nuclear antigen 2 (EBNA2) and activated notch1 transactivate genes by interacting with the cellular protein RBP-J kappa. *Immunobiology*, 198(1-3):299–306, December 1997. ISSN 0171-2985. PMID: 9442401.

[443] G Scala, I Quinto, M R Ruocco, M Mallardo, C Ambrosino, B Squitieri, P Tassone, and S Venuta. Epstein-barr virus nuclear antigen 2 transactivates the long terminal repeat of human immunodeficiency virus type 1. *Journal of virology*, 67(5):2853–2861, May 1993. ISSN 0022-538X. PMID: 8386279 PMCID: PMC237610.

[444] B Ensoli, P Lusso, F Schachter, S F Josephs, J Rappaport, F Negro, R C Gallo, and F Wong-Staal. Human herpes virus-6 increases HIV-1 expression in co-infected t cells via nuclear factors binding to the HIV-1 enhancer. *The EMBO Journal*, 8(10):3019–3027, October 1989. ISSN 0261-4189. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC401379/. PMID: 2573513 PMCID: PMC401379.

[445] J V McCarthy, J Ni, and V M Dixit. RIP2 is a novel NF-kappaB-activating and cell death-inducing kinase. *The Journal of Biological Chemistry*, 273(27):16968–16975, July 1998. ISSN 0021-9258.

[446] Khalid Hussain Bhat, Chinta Krishna Chaitanya, Nazia Parveen, Raja Varman, Sudip Ghosh, and Sangita Mukhopadhyay. Proline-proline-glutamic acid (PPE) protein rv1168c of mycobacterium tuberculosis augments transcription from HIV-1 long terminal repeat promoter. *The Journal of biological chemistry*, 287(20):16930–16946, May 2012. ISSN 1083-351X. doi: 10.1074/jbc.M111.327825. PMID: 22427668 PMCID: PMC3351301.

[447] Y Zhang, K Nakata, M Weiden, and W N Rom. Mycobacterium tuberculosis enhances human immunodeficiency virus-1 replication by transcriptional activation at the long terminal repeat. *The Journal of clinical investigation*, 95(5):2324–2331, May 1995. ISSN 0021-9738. doi: 10.1172/JCI117924. PMID: 7738195 PMCID: PMC295846.

[448] Ozlem Equils, Marco L. Schito, Hiase Karahashi, Zeynep Madak, Ayse Yarali, Kathrin S. Michelsen, Alan Sher, and Moshe Arditi. Toll-like receptor 2 (TLR2) and TLR9 signaling results in HIV-Long terminal repeat trans-activation and HIV replication in HIV-1 transgenic mouse spleen cells: Implications of simultaneous activation of TLRs on HIV replication. *The Journal of Immunology*, 170(10):5159–5164, May 2003. ISSN 0022-1767, 1550-6606. URL http://www.jimmunol.org/content/170/10/5159. PMID: 12734363.

[449] Adrienne Chen, Ian C Boulton, Jodi Pongoski, Alan Cochrane, and Scott D Gray-Owen. Induction of HIV-1 long terminal repeat-mediated transcription by neisseria gonorrhoeae. *AIDS (London, England)*, 17(4):625–628, March 2003. ISSN 0269-9370. doi: 10.1097/01.aids.0000050840.06065.35. PMID: 12598784.

[450] Jian Ding, Aprille Rapista, Natalia Teleshova, Goar Mosoyan, Gary A. Jarvis, Mary E. Klotman, and Theresa L. Chang. Neisseria gonorrhoeae enhances HIV-1 infection of primary resting CD4+ t cells through TLR2 activation. *The Journal of Immunology*, 184(6):2814–2824, March 2010. ISSN 0022-1767, 1550-6606. doi: 10.4049/jimmunol.0902125. URL http://www.jimmunol.org/content/184/6/2814. PMID: 20147631.

[451] Jizhong Zhang, Geling Li, Andre Bafica, Milica Pantelic, Pei Zhang, Hal Broxmeyer, Ying Liu, Lee Wetzler, Johnny J. He, and Tie Chen. Neisseria gonorrhoeae enhances infection of dendritic cells by HIV type 1. *The Journal*

of Immunology, 174(12):7995–8002, June 2005. ISSN 0022-1767, 1550-6606. URL http://www.jimmunol.org/content/174/12/7995. PMID: 15944306.

[452] T S Harrison, S Nong, and S M Levitz. Induction of human immunodeficiency virus type 1 expression in monocytic cells by cryptococcus neoformans and candida albicans. *The Journal of infectious diseases*, 176(2):485–491, August 1997. ISSN 0022-1899. PMID: 9237716.

[453] R. T. Gazzinelli, A. Sher, A. Cheever, S. Gerstberger, M. A. Martin, and P. Dickie. Infection of human immunodeficiency virus 1 transgenic mice with toxoplasma gondii stimulates proviral transcription in macrophages in vivo. *The Journal of Experimental Medicine*, 183(4):1645–1655, April 1996. ISSN 0022-1007, 1540-9538. doi: 10.1084/jem.183.4.1645. URL http://jem.rupress.org/content/183/4/1645. PMID: 8666922.

[454] Emily S Wires, David Alvarez, Curtis Dobrowolski, Yun Wang, Marisela Morales, Jonathan Karn, and Brandon K Harvey. Methamphetamine activates nuclear factor kappa-light-chain-enhancer of activated b cells (NF-B) and induces human immunodeficiency virus (HIV) transcription in human microglial cells. *Journal of neurovirology*, 18(5):400–410, October 2012. ISSN 1538-2443. doi: 10.1007/s13365-012-0103-4. PMID: 22618514 PMCID: PMC3469781.

[455] Maria Teresa Sciortino, Maria Antonietta Medici, Francesca Marino-Merlo, Daniela Zaccaria, Maria Giuffr-Cuculletto, Assunta Venuti, Sandro Grelli, Placido Bramanti, and Antonio Mastino. Involvement of gd/hvem interaction in nf-kb-dependent inhibition of apoptosis by hsv-1 gd. *Biochem Pharmacol*, 76(11):1522–1532, Dec 2008. doi: 10.1016/j.bcp.2008.07.030. URL http://dx.doi.org/10.1016/j.bcp.2008.07.030.

[456] Kari L. Roberts and Joel D. Baines. UL31 of herpes simplex virus 1 is necessary for optimal NF-kappaB activation and expression of viral gene products. *Journal of Virology*, 85(10):4947–4953, May 2011. ISSN 1098-5514. doi: 10.1128/JVI.00068-11.

[457] Steve W. Cole, Yael D. Korin, John L. Fahey, and Jerome A. Zack. Norepinephrine accelerates HIV replication via protein kinase a-dependent effects on cytokine production. *The Journal of Immunology*, 161(2):610–616, July 1998. ISSN 0022-1767, 1550-6606. URL http://www.jimmunol.org/content/161/2/610.

[458] Melissa Kane, Shalini S. Yadav, Julia Bitzegeio, Sebla B. Kutluay, Trinity Zang, Sam J. Wilson, John W. Schoggins, Charles M. Rice, Masahiro Yamashita, Theodora Hatziioannou, and Paul D. Bieniasz. MX2 is an interferon-induced inhibitor of HIV-1 infection. *Nature*, 502(7472):563–566, October 2013. ISSN 0028-0836. doi: 10.1038/nature12653. URL http://www.nature.com.libproxy.ucl.ac.uk/nature/journal/v502/n7472/full/nature12653.html.

[459] Stephen D. Barr, James R. Smiley, and Frederic D. Bushman. The interferon response inhibits HIV particle production by induction of TRIM22. *PLoS Pathog*, 4(2):e1000007, February 2008. doi: 10.1371/journal.ppat.1000007. URL http://dx.plos.org/10.1371/journal.ppat.1000007.

[460] R. Todd Allen, William J. Hunter III, and Devendra K. Agrawal. Morphological and biochemical characterization and analysis of apoptosis. *Journal of Pharmacological and Toxicological Methods*, 37(4):215–228, June 1997. ISSN 1056-8719. doi: 10.1016/S1056-8719(97)00033-6. URL http://www.sciencedirect.com/science/article/pii/S1056871997000336.

[461] Peter Norberg, Shaun Tyler, Alberto Severini, Rich Whitley, Jan-ke Liljeqvist, and Tomas Bergstrm. A genome-wide comparative evolutionary analysis of herpes simplex virus type 1 and varicella zoster virus. *PLoS ONE*, 6(7):e22527, July 2011. doi: 10.1371/journal.pone.0022527. URL http://dx.doi.org/10.1371/journal.pone.0022527.

[462] Thomas W. Berngruber, Franz J. Weissing, and Sylvain Gandon. Inhibition of superinfection and the evolution of viral latency. *Journal of Virology*, 84(19):10200–10208, October 2010. ISSN 0022-538X, 1098-5514. doi: 10.1128/JVI.00865-10. URL http://jvi.asm.org/content/84/19/10200.

[463] Erin Chung and Jonathan Sen. The ongoing pursuit of a prophylactic HSV vaccine. *Reviews in Medical Virology*, 22(5):285–300, September 2012. ISSN 1099-1654. doi: 10.1002/rmv.1709.

[464] A M Colberg-Poley, H C Isom, and F Rapp. Reactivation of herpes simplex virus type 2 from a quiescent state by human cytomegalovirus. *Proceedings of the National Academy of Sciences of the United States of America*, 76(11):

5948–5951, November 1979. ISSN 0027-8424. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC411770/. PMID: 230494 PMCID: PMC411770.

[465] C. L. Wilcox and E. M. Johnson. Characterization of nerve growth factor-dependent herpes simplex virus latency in neurons in vitro. *Journal of Virology*, 62(2):393–399, February 1988. ISSN 0022-538X, 1098-5514. URL http://jvi.asm.org/content/62/2/393. PMID: 2826804.

[466] Joo T Proena, Heather M Coleman, Viv Connor, Douglas J Winton, and Stacey Efstathiou. A historical analysis of herpes simplex virus promoter activation in vivo reveals distinct populations of latently infected neurones. *The Journal of general virology*, 89(Pt 12):2965–2974, December 2008. ISSN 0022-1317. doi: 10.1099/vir.0.2008/005066-0.

[467] Devis Sinani, Ethan Cordes, Aspen Workman, Prasanth Thunuguntia, and Clinton Jones. Stress-induced cellular transcription factors expressed in trigeminal ganglionic neurons stimulate the herpes simplex virus 1 ICP0 promoter. *Journal of virology*, 87(23):13042–13047, December 2013. ISSN 1098-5514. doi: 10.1128/JVI.02476-13. PMID: 24027338 PMCID: PMC3838111.

[468] Roger D Everett, Marie-Laure Parsy, and Anne Orr. Analysis of the functions of herpes simplex virus type 1 regulatory protein ICP0 that are critical for lytic infection and derepression of quiescent viral genomes. *Journal of virology*, 83(10):4963–4977, May 2009. ISSN 1098-5514. doi: 10.1128/JVI.02593-08. PMID: 19264778 PMCID: PMC2682082.

[469] Xiao-Ping Chen, Jia Li, Marina Mata, James Goss, Darren Wolfe, Joseph C. Glorioso, and David J. Fink. Herpes simplex virus type 1 ICP0 protein does not accumulate in the nucleus of primary neurons in culture. *Journal of Virology*, 74(21):10132–10141, November 2000. ISSN 0022-538X, 1098-5514. doi: 10.1128/JVI.74.21.10132-10141.2000. URL http://jvi.asm.org/content/74/21/10132.

[470] Pascal Lopez, Charles Van Sant, and Bernard Roizman. Requirements for the nuclear-cytoplasmic translocation of infected-cell protein 0 of herpes simplex virus 1. *Journal of Virology*, 75(8):3832–3840, April 2001. ISSN 0022-538X. doi: 10.1128/JVI.75.8.3832-3840.2001. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC114874/. PMID: 11264372 PMCID: PMC114874.

[471] Devin S. Gary and Mark P. Mattson. Integrin signaling via the PI3-kinaseAkt pathway increases neuronal resistance to glutamate-induced apoptosis. *Journal of Neurochemistry*, 76(5):1485–1496, March 2001. ISSN 1471-4159. doi: 10.1046/j.1471-4159.2001.00173.x. URL http://onlinelibrary.wiley.com/doi/10.1046/j.1471-4159.2001.00173.x/abstract.

[472] Harris Perlman, Lisa J. Pagliari, Constantinos Georganas, Toshiaki Mano, Kenneth Walsh, and Richard M. Pope. Flice-inhibitory protein expression during macrophage differentiation confers resistance to FAS-Mediated apoptosis. *The Journal of Experimental Medicine*, 190(11):1679–1688, December 1999. ISSN 0022-1007, 1540-9538. doi: 10.1084/jem.190.11.1679. URL http://jem.rupress.org/content/190/11/1679. PMID: 10587358.

[473] ZaiFang Yu, Hong Luo, Weiming Fu, and Mark P. Mattson. The endoplasmic reticulum stress-responsive protein GRP78 protects neurons against excitotoxicity and apoptosis: Suppression of oxidative stress and stabilization of calcium homeostasis. *Experimental Neurology*, 155(2):302–314, February 1999. ISSN 0014-4886. doi: 10.1006/exnr.1998.7002. URL http://www.sciencedirect.com/science/article/pii/S0014488698970029.

[474] Jong K. Yun, Thomas S. McCormick, Claudia Villabona, Raymond R. Judware, Mara B. Espinosa, and Eduardo G. Lapetina. Inflammatory mediators are perpetuated in macrophages resistant to apoptosis induced by hypoxia. *Proceedings of the National Academy of Sciences*, 94(25):13903–13908, December 1997. ISSN 0027-8424, 1091-6490. URL http://www.pnas.org/content/94/25/13903.

[475] Gaelle Kolb and Thomas M Kristie. Association of the cellular coactivator HCF-1 with the golgi apparatus in sensory neurons. *Journal of virology*, 82(19):9555–9563, October 2008. ISSN 1098-5514. doi: 10.1128/JVI.01174-08.

[476] B Levine, Q Huang, J T Isaacs, J C Reed, D E Griffin, and J M Hardwick. Conversion of lytic to persistent alphavirus infection by the bcl-2 cellular oncogene. *Nature*, 361(6414):739–742, February 1993. ISSN 0028-0836. doi: 10.1038/361739a0.

[477] B. Wigdahl, C. A. Smith, H. M. Traglia, and F. Rapp. Herpes simplex virus latency in isolated human neurons. *Proceedings of the National Academy of Sciences*, 81(19):6217–6221, October 1984. ISSN 0027-8424, 1091-6490. URL http://www.pnas.org/content/81/19/6217. PMID: 6091142.

[478] A M Deatly, J G Spivack, E Lavi, and N W Fraser. RNA from an immediate early region of the type 1 herpes simplex virus genome is present in the trigeminal ganglia of latently infected mice. *Proceedings of the National Academy of Sciences of the United States of America*, 84(10):3204–3208, May 1987. ISSN 0027-8424. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC304837/.

[479] J. G. Spivack and N. W. Fraser. Detection of herpes simplex virus type 1 transcripts during latent infection in mice. *Journal of Virology*, 61(12):3841–3847, December 1987. ISSN 0022-538X.

[480] J. G. Stevens, E. K. Wagner, G. B. Devi-Rao, M. L. Cook, and L. T. Feldman. RNA complementary to a herpesvirus alpha gene mRNA is prominent in latently infected neurons. *Science (New York, N.Y.)*, 235(4792): 1056–1059, February 1987. ISSN 0036-8075.

[481] I Steiner, J G Spivack, R P Lirette, S M Brown, A R MacLean, J H Subak-Sharpe, and N W Fraser. Herpes simplex virus type 1 latency-associated transcripts are evidently not essential for latent infection. *The EMBO Journal*, 8 (2):505–511, February 1989. ISSN 0261-4189. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC400833/.

[482] D. A. Garber, P. A. Schaffer, and D. M. Knipe. A LAT-associated function reduces productive-cycle gene expression during acute infection of murine sensory neurons with herpes simplex virus type 1. *Journal of Virology*, 71(8):5885–5893, August 1997. ISSN 0022-538X.

[483] Nurith Mador, Daniel Goldenberg, Oren Cohen, Amos Panet, and Israel Steiner. Herpes simplex virus type 1 latency-associated transcripts suppress viral replication and reduce immediate-early gene mRNA levels in a neuronal cell line. *Journal of Virology*, 72(6):5067–5075, June 1998. ISSN 0022-538X, 1098-5514. URL http://jvi.asm.org/content/72/6/5067.

[484] Kerry K. Brinkman, Prakhar Mishra, and Nigel W. Fraser. The half-life of the HSV-1 1.5 kb LAT intron is similar to the half-life of the 2.0 kb LAT intron. *Journal of neurovirology*, 19(1):102–108, February 2013. ISSN 1355-0284. doi: 10.1007/s13365-012-0146-6. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3568251/.

[485] J. M. Zabolotny, C. Krummenacher, and N. W. Fraser. The herpes simplex virus type 1 2.0-kilobase latency-associated transcript is a stable intron which branches at a guanosine. *Journal of Virology*, 71(6):4199–4208, June 1997. ISSN 0022-538X.

[486] Angus C Wilson and Ian Mohr. A cultured affair: HSV latency and reactivation in neurons. *Trends in microbiology*, 20(12):604–611, December 2012. ISSN 1878-4380. doi: 10.1016/j.tim.2012.08.005. PMID: 22963857.

[487] Craig S. Miller, Robert J. Danaher, and Robert J. Jacob. ICP0 is not required for efficient stress-induced reactivation of herpes simplex virus type 1 from cultured quiescently infected neuronal cells. *Journal of Virology*, 80(7):3360–3368, April 2006. ISSN 0022-538X, 1098-5514. doi: 10.1128/JVI.80.7.3360-3368.2006. URL http://jvi.asm.org/content/80/7/3360. PMID: 16537603.

[488] T. L. Kielian and F. Blecha. CD14 and other recognition molecules for lipopolysaccharide: a review. *Immunopharmacology*, 29(3):187–205, April 1995. ISSN 0162-3109.

[489] Charles H. Cook, Joanne Trgovcich, Peter D. Zimmerman, Yingxue Zhang, and Daniel D. Sedmak. Lipopolysaccharide, tumor necrosis factor alpha, or interleukin-1? triggers reactivation of latent cytomegalovirus in immunocompetent mice. *Journal of Virology*, 80(18):9151–9158, September 2006. ISSN 0022-538X. doi: 10.1128/JVI.00216-06. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1563908/.

[490] Timothy Block, Shawn Barney, John Masonis, John Maggioncalda, Tibor Valyi-Nagy, and Nigel W. Fraser. Long term herpes simplex virus type 1 infection of nerve growth factor-treated PC12 cells. *Journal of General Virology*, 75(9):2481–2487, September 1994. ISSN 0022-1317, 1465-2099. doi: 10.1099/0022-1317-75-9-2481. URL http://vir.sgmjournals.org/content/75/9/2481. PMID: 8077952.

[491] Elizabeth A. Hunsperger and Christine L. Wilcox. Caspase-3-dependent reactivation of latent herpes simplex virus type 1 in sensory neuronal cultures. *Journal of Neurovirology*, 9(3):390–398, June 2003. ISSN 1355-0284. doi: 10.1080/13550280390201678.

[492] R J Danaher, R J Jacob, and C S Miller. Establishment of a quiescent herpes simplex virus type 1 infection in neurally-differentiated PC12 cells. *Journal of neurovirology*, 5(3):258–267, June 1999. ISSN 1355-0284. PMID: 10414516.

[493] R. H. Alasbahi and M. F. Melzig. Forskolin and derivatives as tools for studying the role of cAMP. *Die Pharmazie*, 67(1):5–13, January 2012. ISSN 0031-7144.

[494] Craig Miller, Partha Bhattacharjee, Shiro Higaki, Robert Jacob, Robert Danaher, Hilary Thompson, and James Hill. Herpesvirus quiescence (QIF) in neuronal cells VI: Correlative analysis demonstrates usefulness of QIF-PC12 cells to examine HSV-1 latency, reactivation and genes implicated in its regulation. *Current Eye Research*, 26(3-4): 239–248, April 2003. ISSN 0271-3683.

[495] Nurith Mador, Amos Panet, and Israel Steiner. The latency-associated gene of herpes simplex virus type 1 (HSV-1) interferes with superinfection by HSV-1. *Journal of neurovirology*, 8 Suppl 2:97–102, December 2002. ISSN 1355-0284. doi: 10.1080/13550280290167920. PMID: 12491159.

[496] Sariah J. Allen, Antje Rhode-Kurnow, Kevin R. Mott, Xianzhi Jiang, Dale Carpenter, J. Ignacio Rodriguez-Barbosa, Clinton Jones, Steven L. Wechsler, Carl F. Ware, and Homayon Ghiasi. Interactions between herpesvirus entry mediator (TNFRSF14) and latency-associated transcript during herpes simplex virus 1 latency. *Journal of Virology*, 88(4):1961–1971, February 2014. ISSN 1098-5514. doi: 10.1128/JVI.02467-13.

[497] Leticia Frizzo da Silva and Clinton Jones. Small non-coding RNAs encoded within the herpes simplex virus type 1 latency associated transcript (LAT) cooperate with the retinoic acid inducible gene i (RIG-i) to induce beta-interferon promoter activity and promote cell survival. *Virus Research*, 175(2):101–109, August 2013. ISSN 1872-7492. doi: 10.1016/j.virusres.2013.04.005.

[498] M. P. Nicoll, J. T. Proena, V. Connor, and S. Efstathiou. Influence of herpes simplex virus 1 latency-associated transcripts on the establishment and maintenance of latency in the ROSA26r reporter mouse model. *Journal of Virology*, 86(16):8848–8858, August 2012. ISSN 1098-5514. doi: 10.1128/JVI.00652-12.

[499] Frdric Catez, Antoine Rousseau, Marc Labetoulle, and Patrick Lomonte. Detection of the genome and transcripts of a persistent DNA virus in neuronal tissues by fluorescent in situ hybridization combined with immunostaining. *Journal of Visualized Experiments: JoVE*, (83):e51091, 2014. ISSN 1940-087X. doi: 10.3791/51091.

[500] C L Wilcox, L S Crnic, and L I Pizer. Replication, latent infection, and reactivation in neuronal culture with a herpes simplex virus thymidine kinase-negative mutant. *Virology*, 187(1):348–352, March 1992. ISSN 0042-6822. PMID: 1310559.

[501] B L Wigdahl, R J Ziegler, M Sneve, and F Rapp. Herpes simplex virus latency and reactivation in isolated rat sensory neurons. *Virology*, 127(1):159–167, May 1983. ISSN 0042-6822. PMID: 6305013.

[502] C. L. Wilcox and E. M. Johnson. Nerve growth factor deprivation results in the reactivation of latent herpes simplex virus in vitro. *Journal of Virology*, 61(7):2311–2315, July 1987. ISSN 0022-538X, 1098-5514. URL `http://jvi.asm.org/content/61/7/2311`. PMID: 3035230.

[503] Joanna L. Miller and E. Margot Anders. Virus-cell interactions in the induction of type 1 interferon by influenza virus in mouse spleen cells. *J Gen Virol*, 84(1):193–202, January 2003. doi: 10.1099/vir.0.18590-0.

[504] Ina Hogk, Michaela Kaufmann, Doris Finkelmeier, Steffen Rupp, and Anke Burger-Kentischer. An in vitro HSV-1 reactivation model containing quiescently infected PC12 cells. *BioResearch open access*, 2(4):250–257, August 2013. ISSN 2164-7844. doi: 10.1089/biores.2013.0019. PMID: 23914331 PMCID: PMC3731678.

[505] M K Isaacson, L K Juckem, and T Compton. Virus entry and innate immune activation. *Current Topics in Microbiology and Immunology*, 325:85–100, 2008. ISSN 0070-217X.

[506] David Hare and Karen L. Mossman. Novel paradigms of innate immune sensing of viral infections. *Cytokine*, 63 (3):219–224, September 2013. ISSN 1096-0023. doi: 10.1016/j.cyto.2013.06.001.

[507] Christian K. Holm, Sren B. Jensen, Martin R. Jakobsen, Natalia Cheshenko, Kristy A. Horan, Hanne B. Moeller, Regina Gonzalez-Dosal, Simon B. Rasmussen, Maria H. Christensen, Timur O. Yarovinsky, Frazer J. Rixon, Betsy C. Herold, Katherine A. Fitzgerald, and Sren R. Paludan. Virus-cell fusion as a trigger of innate immunity dependent on the adaptor STING. *Nature Immunology*, 13(8):737–743, August 2012. ISSN 1529-2916. doi: 10.1038/ni.2350.

[508] Amy Andrew and Klaus Strebel. HIV-1 accessory proteins: Vpu and vif. *Methods in Molecular Biology (Clifton, N.J.)*, 1087:135–158, 2014. ISSN 1940-6029. doi: 10.1007/978-1-62703-670-2_12.

[509] E. A. Cohen, E. F. Terwilliger, Y. Jalinoos, J. Proulx, J. G. Sodroski, and W. A. Haseltine. Identification of HIV-1 vpr product and function. *Journal of Acquired Immune Deficiency Syndromes*, 3(1):11–18, 1990. ISSN 0894-9255.

[510] D H Gabuzda, K Lawrence, E Langhoff, E Terwilliger, T Dorfman, W A Haseltine, and J Sodroski. Role of vif in replication of human immunodeficiency virus type 1 in CD4+ t lymphocytes. *Journal of Virology*, 66(11): 6489–6495, November 1992. ISSN 0022-538X. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC240141/.

[511] D. H. Gabuzda, H. Li, K. Lawrence, B. S. Vasir, K. Crawford, and E. Langhoff. Essential role of vif in establishing productive HIV-1 infection in peripheral blood t lymphocytes and monocyte/macrophages. *Journal of Acquired Immune Deficiency Syndromes*, 7(9):908–915, September 1994. ISSN 0894-9255.

[512] Ann M. Sheehy, Nathan C. Gaddis, and Michael H. Malim. The antiretroviral enzyme APOBEC3g is degraded by the proteasome in response to HIV-1 vif. *Nature Medicine*, 9(11):1404–1407, November 2003. ISSN 1078-8956. doi: 10.1038/nm945.

[513] Sandra Kao, Mohammad A. Khan, Eri Miyagi, Ron Plishka, Alicia Buckler-White, and Klaus Strebel. The human immunodeficiency virus type 1 vif protein reduces intracellular expression and inhibits packaging of APOBEC3g (CEM15), a cellular inhibitor of virus infectivity. *Journal of Virology*, 77(21):11398–11407, November 2003. ISSN 0022-538X, 1098-5514. doi: 10.1128/JVI.77.21.11398-11407.2003. URL http://jvi.asm.org/content/77/21/11398.

[514] James H. Miller, Vlad Presnyak, and Harold C. Smith. The dimerization domain of HIV-1 viral infectivity factor vif is required to block virion incorporation of APOBEC3g. *Retrovirology*, 4:81, 2007. ISSN 1742-4690. doi: 10.1186/1742-4690-4-81.

[515] J W Balliet, D L Kolson, G Eiger, F M Kim, K A McGann, A Srinivasan, and R Collman. Distinct effects in primary macrophages and lymphocytes of the human immunodeficiency virus type 1 accessory genes vpr, vpu, and nef: mutational analysis of a primary HIV-1 isolate. *Virology*, 200(2):623–631, May 1994. ISSN 0042-6822. doi: 10.1006/viro.1994.1225.

[516] R. I. Connor, B. K. Chen, S. Choe, and N. R. Landau. Vpr is required for efficient replication of human immunodeficiency virus type-1 in mononuclear phagocytes. *Virology*, 206(2):935–944, February 1995. ISSN 0042-6822. doi: 10.1006/viro.1995.1016.

[517] L. Wang, S. Mukherjee, F. Jia, O. Narayan, and L. J. Zhao. Interaction of virion protein vpr of human immunodeficiency virus type 1 with cellular transcription factor sp1 and trans-activation of viral long terminal repeat. *The Journal of Biological Chemistry*, 270(43):25564–25569, October 1995. ISSN 0021-9258.

[518] Shigeki Hoshino, Mitsuru Konishi, Masako Mori, Mari Shimura, Chiaki Nishitani, Yoshio Kuroki, Yoshio Koyanagi, Shigeyuki Kano, Hiroyuki Itabe, and Yukihito Ishizaka. HIV-1 vpr induces TLR4/MyD88-mediated IL-6 production and reactivates viral production from latency. *Journal of Leukocyte Biology*, 87(6):1133–1143, June 2010. ISSN 1938-3673. doi: 10.1189/jlb.0809547.

[519] Dool-Bboon Kim and Neal A. DeLuca. Phosphorylation of transcription factor sp1 during herpes simplex virus type 1 infection. *Journal of Virology*, 76(13):6473–6479, July 2002. ISSN 0022-538X. doi: 10.1128/JVI.76.13.6473-6479.2002. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC136260/.

[520] Amei Deng, Chao Chen, Yukihito Ishizaka, Xinwen Chen, Binlian Sun, and Rongge Yang. Human immunodeficiency virus type 1 vpr increases hepatitis c virus RNA replication in cell culture. *Virus Research*, 184:93–102, May 2014. ISSN 1872-7492. doi: 10.1016/j.virusres.2014.02.017.

[521] E. Jacotot, K. F. Ferri, C. El Hamel, C. Brenner, S. Druillennec, J. Hoebeke, P. Rustin, D. Mtivier, C. Lenoir, M. Geuskens, H. L. Vieira, M. Loeffler, A. S. Belzacq, J. P. Briand, N. Zamzami, L. Edelman, Z. H. Xie, J. C. Reed, B. P. Roques, and G. Kroemer. Control of mitochondrial membrane permeabilization by adenine nucleotide translocator interacting with HIV-1 viral protein rR and bcl-2. *The Journal of Experimental Medicine*, 193(4): 509–519, February 2001. ISSN 0022-1007.

[522] Hiroko Kitayama, Yoshiharu Miura, Yoshinori Ando, Shigeki Hoshino, Yukihito Ishizaka, and Yoshio Koyanagi. Human immunodeficiency virus type 1 vpr inhibits axonal outgrowth through induction of mitochondrial dysfunction. *Journal of Virology*, 82(5):2528–2542, March 2008. ISSN 1098-5514. doi: 10.1128/JVI.02094-07.

[523] Jiangfang Wang, Emma L. Reuschel, Jason M. Shackelford, Lauren Jeang, Debra K. Shivers, J. Alan Diehl, Xiao-Fang Yu, and Terri H. Finkel. HIV-1 vif promotes the g?- to s-phase cell-cycle transition. *Blood*, 117(4):1260–1269, January 2011. ISSN 1528-0020. doi: 10.1182/blood-2010-06-289215.

[524] Keiko Sakai, R. Anthony Barnitz, Benjamin Chaigne-Delalande, Nicolas Bidre, and Michael J. Lenardo. Human immunodeficiency virus type 1 vif causes dysfunction of cdk1 and CyclinB1: implications for cell cycle arrest. *Virology Journal*, 8:219, 2011. ISSN 1743-422X. doi: 10.1186/1743-422X-8-219.

[525] E. Jacotot, L. Ravagnan, M. Loeffler, K. F. Ferri, H. L. Vieira, N. Zamzami, P. Costantini, S. Druillennec, J. Hoebeke, J. P. Briand, T. Irinopoulou, E. Daugas, S. A. Susin, D. Cointe, Z. H. Xie, J. C. Reed, B. P. Roques, and G. Kroemer. The HIV-1 viral protein r induces apoptosis via a direct effect on the mitochondrial permeability transition pore. *The Journal of Experimental Medicine*, 191(1):33–46, January 2000. ISSN 0022-1007.

[526] J. B. Jowett, V. Planelles, B. Poon, N. P. Shah, M. L. Chen, and I. S. Chen. The human immunodeficiency virus type 1 vpr gene arrests infected t cells in the g2 + m phase of the cell cycle. *Journal of Virology*, 69(10):6304–6313, October 1995. ISSN 0022-538X.

[527] M. E. Rogel, L. I. Wu, and M. Emerman. The human immunodeficiency virus type 1 vpr gene prevents cell proliferation during chronic infection. *Journal of Virology*, 69(2):882–888, February 1995. ISSN 0022-538X.

[528] Anke Heigele, Daniel Sauter, Jan Mnch, and Frank Kirchhoff. HIV-1 accessory proteins: Nef. *Methods in Molecular Biology (Clifton, N.J.)*, 1087:115–123, 2014. ISSN 1940-6029. doi: 10.1007/978-1-62703-670-2_10.

[529] Scott D. Briggs, Beata Scholtz, Jean-Marc Jacque, Simon Swingler, Mario Stevenson, and Thomas E. Smithgall. HIV-1 nef promotes survival of myeloid cells by a stat3-dependent pathway. *Journal of Biological Chemistry*, 276(27):25605–25611, July 2001. ISSN 0021-9258, 1083-351X. doi: 10.1074/jbc.M103244200. URL http://www.jbc.org/content/276/27/25605.

[530] Eleonora Olivetta and Maurizio Federico. HIV-1 nef protects human-monocyte-derived macrophages from HIV-1-induced apoptosis. *Experimental Cell Research*, 312(6):890–900, April 2006. ISSN 0014-4827. doi: 10.1016/j.yexcr.2005.12.003.

[531] W. Abbas, K. A. Khan, A. Kumar, M. K. Tripathy, I. Dichamp, M. Keita, U. Mahlknecht, O. Rohr, and G. Herbein. Blockade of BFA-mediated apoptosis in macrophages by the HIV-1 nef protein. *Cell Death & Disease*, 5(2):e1080, February 2014. doi: 10.1038/cddis.2014.16.

[532] Karuppiah Muthumani, Daniel S. Hwang, Andrew Y. Choo, Shanmugam Mayilvahanan, Nathanael S. Dayes, Khanh P. Thieu, and David B. Weiner. HIV-1 vpr inhibits the maturation and activation of macrophages and dendritic cells in vitro. *International Immunology*, 17(2):103–116, February 2005. ISSN 0953-8178. doi: 10.1093/intimm/dxh190.

[533] R. Geleziunas, W. Xu, K. Takeda, H. Ichijo, and W. C. Greene. HIV-1 nef inhibits ASK1-dependent death signalling providing a potential mechanism for protecting the infected host cell. *Nature*, 410(6830):834–838, April 2001. ISSN 0028-0836. doi: 10.1038/35071111.

[534] D. Wolf, V. Witte, B. Laffert, K. Blume, E. Stromer, S. Trapp, P. d'Aloja, A. Schrmann, and A. S. Baur. HIV-1 nef associated PAK and PI3-kinases stimulate akt-independent bad-phosphorylation to induce anti-apoptotic signals. *Nature Medicine*, 7(11):1217–1224, November 2001. ISSN 1078-8956. doi: 10.1038/nm1101-1217.

[535] Tram N. Q. Pham, Sabelo Lukhele, Fadi Hajjar, Jean-Pierre Routy, and ric A. Cohen. HIV nef and vpu protect HIV-infected CD4+ t cells from antibody-mediated cell lysis through down-modulation of CD4 and BST2. *Retrovirology*, 11:15, 2014. ISSN 1742-4690. doi: 10.1186/1742-4690-11-15.

[536] R. L. Thompson and N. M. Sawtell. Herpes simplex virus type 1 latency-associated transcript gene promotes neuronal survival. *Journal of Virology*, 75(14):6660–6675, July 2001. ISSN 0022-538X, 1098-5514. doi: 10.1128/JVI.75.14.6660-6675.2001. URL http://jvi.asm.org/content/75/14/6660.

[537] G. C. Perng, C. Jones, J. Ciacci-Zanella, M. Stone, G. Henderson, A. Yukht, S. M. Slanina, F. M. Hofman, H. Ghiasi, A. B. Nesburn, and S. L. Wechsler. Virus-induced neuronal apoptosis blocked by the herpes simplex virus latency-associated transcript. *Science*, 287(5457):1500–1503, Feb 2000.

[538] G.-C. Perng, B. Maguen, L. Jin, K.R. Mott, N. Osorio, S.M. Slanina, A. Yukht, H. Ghiasi, A.B. Nesburn, M. Inman, G. Henderson, C. Jones, and S.L. Wechsler. A gene capable of blocking apoptosis can substitute for the herpes simplex virus type 1 latency-associated transcript gene and restore wild-type reactivation levels. *Journal of Virology*, 76(3):1224–1235, 2002. ISSN 0022-538X.

[539] T. E. Biggs, S. J. Cooke, C. H. Barton, M. P. Harris, K. Saksela, and D. A. Mann. Induction of activator protein 1 (AP-1) in macrophages by human immunodeficiency virus type-1 NEF is a cell-type-specific response that requires both hck and MAPK signaling events. *Journal of Molecular Biology*, 290(1):21–35, July 1999. ISSN 0022-2836. doi: 10.1006/jmbi.1999.2849.

[540] M. Federico, Z. Percario, E. Olivetta, G. Fiorucci, C. Muratori, A. Micheli, G. Romeo, and E. Affabris. HIV-1 nef activates STAT1 in human monocytes/macrophages through the release of soluble factors. *Blood*, 98(9): 2752–2761, November 2001. ISSN 0006-4971.

[541] Zulema Percario, Eleonora Olivetta, Gianna Fiorucci, Giorgio Mangino, Silvia Peretti, Giovanna Romeo, Elisabetta Affabris, and Maurizio Federico. Human immunodeficiency virus type 1 (HIV-1) nef activates STAT3 in primary human monocyte/macrophages through the release of soluble factors: involvement of nef domains interacting with the cell endocytotic machinery. *Journal of Leukocyte Biology*, 74(5):821–832, November 2003. ISSN 0741-5400. doi: 10.1189/jlb.0403161.

[542] Gary D. Luker, J. Patrick Bardill, Julie L. Prior, Christina M. Pica, David Piwnica-Worms, and David A. Leib. Noninvasive bioluminescence imaging of herpes simplex virus type 1 infection and therapy in living mice. *Journal of Virology*, 76(23):12149–12161, December 2002. ISSN 0022-538X. doi: 10.1128/JVI.76.23.12149-12161.2002. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC136903/.

[543] Tal Kramer and Lynn W. Enquist. Directional spread of alphaherpesviruses in the nervous system. *Viruses*, 5(2): 678–707, February 2013. ISSN 1999-4915. doi: 10.3390/v5020678. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3640521/. PMID: 23435239 PMCID: PMC3640521.

[544] Guoying Zhou, Te Du, and Bernard Roizman. The role of the CoREST/REST repressor complex in herpes simplex virus 1 productive infection and in latency. *Viruses*, 5(5):1208–1218, April 2013. ISSN 1999-4915. doi: 10.3390/v5051208. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3712303/. PMID: 23628827 PMCID: PMC3712303.

[545] Haidong Gu, Yu Liang, Gail Mandel, and Bernard Roizman. Components of the REST/CoREST/histone deacetylase repressor complex are disrupted, modified, and translocated in HSV-1-infected cells. *Proceedings of the National Academy of Sciences of the United States of America*, 102(21):7571–7576, May 2005. ISSN 0027-8424, 1091-6490. doi: 10.1073/pnas.0502658102. URL http://www.pnas.org/content/102/21/7571. PMID: 15897453.

[546] T Gerster and R G Roeder. A herpesvirus trans-activating protein interacts with transcription factor OTF-1 and other cellular proteins. *Proceedings of the National Academy of Sciences of the United States of America*, 85(17):

6347–6351, September 1988. ISSN 0027-8424. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC281967/. PMID: 2842768 PMCID: PMC281967.

[547] T M Kristie, J H LeBowitz, and P A Sharp. The octamer-binding proteins form multi-protein–DNA complexes with the HSV alpha TIF regulatory protein. *The EMBO Journal*, 8(13):4229–4238, December 1989. ISSN 0261-4189. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC401620/. PMID: 2556266 PMCID: PMC401620.

[548] S Stern, M Tanaka, and W Herr. The oct-1 homoeodomain directs formation of a multiprotein-DNA complex with the HSV transactivator VP16. *Nature*, 341(6243):624–630, October 1989. ISSN 0028-0836. doi: 10.1038/341624a0. PMID: 2571937.

[549] C M apRhys, D M Ciufo, E A O'Neill, T J Kelly, and G S Hayward. Overlapping octamer and TAATGARAT motifs in the VF65-response elements in herpes simplex virus immediate-early promoters represent independent binding sites for cellular nuclear factor III. *Journal of Virology*, 63(6):2798–2812, June 1989. ISSN 0022-538X. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC250783/. PMID: 2542590 PMCID: PMC250783.

[550] Haidong Gu and Bernard Roizman. Herpes simplex virus-infected cell protein 0 blocks the silencing of viral DNA by dissociating histone deacetylases from the CoRESTREST complex. *Proceedings of the National Academy of Sciences*, 104(43):17134–17139, October 2007. ISSN 0027-8424, 1091-6490. doi: 10.1073/pnas.0707266104. URL http://www.pnas.org/content/104/43/17134. PMID: 17939992.

[551] Betty Poon and Irvin S. Y. Chen. Human immunodeficiency virus type 1 (HIV-1) vpr enhances expression from unintegrated HIV-1 DNA. *Journal of Virology*, 77(7):3962–3972, April 2003. ISSN 0022-538X.

[552] Y Fu, N Comella, K Tognazzi, L F Brown, H F Dvorak, and O Kocher. Cloning of DLM-1, a novel gene that is up-regulated in activated macrophages, using RNA differential display. *Gene*, 240(1):157–163, November 1999. ISSN 0378-1119.

[553] Sung Chul Ha, Dong Van Quyen, Hye-Yeon Hwang, Doo-Byoung Oh, Bernard A Brown, Seon Min Lee, Hyun-Ju Park, Jin-Hyun Ahn, Kyeong Kyu Kim, and Yang-Gyun Kim. Biochemical characterization and preliminary x-ray crystallographic study of the domains of human ZBP1 bound to left-handed Z-DNA. *Biochimica Et Biophysica Acta*, 1764(2):320–323, February 2006. ISSN 0006-3002. doi: 10.1016/j.bbapap.2005.12.012.

[554] Stefan Rothenburg, Thomas Schwartz, Friedrich Koch-Nolte, and Friedrich Haag. Complex regulation of the human gene for the Z-DNA binding protein DLM-1. *Nucleic Acids Research*, 30(4):993–1000, February 2002. ISSN 1362-4962.

[555] ZhiChao Wang, Myoung Kwon Choi, Tatsuma Ban, Hideyuki Yanai, Hideo Negishi, Yan Lu, Tomohiko Tamura, Akinori Takaoka, Kazuko Nishikura, and Tadatsugu Taniguchi. Regulation of innate immune responses by DAI (DLM-1/ZBP1) and other DNA-sensing molecules. *Proceedings of the National Academy of Sciences of the United States of America*, 105(14):5477–5482, April 2008. ISSN 0027-8424. doi: 10.1073/pnas.0801295105.

[556] Victor R DeFilippis, David Alvarado, Tina Sali, Stefan Rothenburg, and Klaus Frh. Human cytomegalovirus induces the interferon response via the DNA sensor ZBP1. *Journal of Virology*, 84(1):585–598, January 2010. ISSN 1098-5514. doi: 10.1128/JVI.01748-09.

[557] Kouji Kobiyama, Fumihiko Takeshita, Nao Jounai, Asako Sakaue-Sawano, Atsushi Miyawaki, Ken J Ishii, Taro Kawai, Shin Sasaki, Hisashi Hirano, Norihisa Ishii, Kenji Okuda, and Koichi Suzuki. Extrachromosomal histone H2B mediates innate antiviral immune responses induced by intracellular double-stranded DNA. *Journal of Virology*, 84(2):822–832, January 2010. ISSN 1098-5514. doi: 10.1128/JVI.01339-09.

[558] Juliane Lippmann, Stefan Rothenburg, Nikolaus Deigendesch, Julia Eitel, Karolin Meixenberger, Vincent van Laak, Hortense Slevogt, Philippe Dje N'guessan, Stefan Hippenstiel, Trinad Chakraborty, Antje Flieger, Norbert Suttorp, and Bastian Opitz. IFNbeta responses induced by intracellular bacteria or cytosolic DNA in different human cells do not require ZBP1 (DLM-1/DAI). *Cellular Microbiology*, 10(12):2579–2588, December 2008. ISSN 1462-5822. doi: 10.1111/j.1462-5822.2008.01232.x.

[559] K L DeYoung, M E Ray, Y A Su, S L Anzick, R W Johnstone, J A Trapani, P S Meltzer, and J M Trent. Cloning a

novel member of the human interferon-inducible gene family associated with control of tumorigenicity in a model of human melanoma. *Oncogene*, 15(4):453–457, July 1997. ISSN 0950-9232. doi: 10.1038/sj.onc.1201206.

[560] Veit Hornung, Andrea Ablasser, Marie Charrel-Dennis, Franz Bauernfeind, Gabor Horvath, Daniel R Caffrey, Eicke Latz, and Katherine A Fitzgerald. AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. *Nature*, 458(7237):514–518, March 2009. ISSN 1476-4687. doi: 10.1038/nature07725.

[561] Tilmann Burckstummer, Christoph Baumann, Stephan Bluml, Evelyn Dixit, Gerhard Durnberger, Hannah Jahn, Melanie Planyavsky, Martin Bilban, Jacques Colinge, Keiryn L Bennett, and Giulio Superti-Furga. An orthogonal proteomic-genomic screen identifies AIM2 as a cytoplasmic DNA sensor for the inflammasome. *Nat Immunol*, 10 (3):266–272, March 2009. ISSN 1529-2908. doi: 10.1038/ni.1702.

[562] Tara L. Roberts, Adi Idris, Jasmyn A. Dunn, Greg M. Kelly, Carol M. Burnton, Samantha Hodgson, Lani L. Hardy, Valerie Garceau, Matthew J. Sweet, Ian L. Ross, David A. Hume, and Katryn J. Stacey. HIN-200 proteins regulate caspase activation in response to foreign cytoplasmic DNA. *Science*, 323(5917):1057 –1060, February 2009. doi: 10.1126/science.1169841.

[563] R Pierini, C Juruj, M Perret, C L Jones, P Mangeot, D S Weiss, and T Henry. AIM2/ASC triggers caspase-8-dependent apoptosis in francisella-infected caspase-1-deficient macrophages. *Cell death and differentiation*, 19(10): 1709–1721, October 2012. ISSN 1476-5403. doi: 10.1038/cdd.2012.51. PMID: 22555457 PMCID: PMC3438500.

[564] Jonathan W Jones, Nobuhiko Kayagaki, Petr Broz, Thomas Henry, Kim Newton, Karen O'Rourke, Salina Chan, Jennifer Dong, Yan Qu, Meron Roose-Girma, Vishva M Dixit, and Denise M Monack. Absent in melanoma 2 is required for innate immune recognition of francisella tularensis. *Proceedings of the National Academy of Sciences of the United States of America*, 107(21):9771–9776, May 2010. ISSN 1091-6490. doi: 10.1073/pnas.1003738107. PMID: 20457908 PMCID: PMC2906881.

[565] Eunjoo Park, Hee Sam Na, Yu-Ri Song, Seong Yeol Shin, You-Me Kim, and Jin Chung. Activation of NLRP3 and AIM2 inflammasomes by porphyromonas gingivalis infection. *Infection and immunity*, 82(1):112–123, January 2014. ISSN 1098-5522. doi: 10.1128/IAI.00862-13. PMID: 24126516 PMCID: PMC3911849.

[566] Yang Yang, Xiangmei Zhou, Mohammed Kouadir, Fushan Shi, Tianjian Ding, Chunfa Liu, Jin Liu, Min Wang, Lifeng Yang, Xiaomin Yin, and Deming Zhao. the AIM2 inflammasome is involved in macrophage activation during infection with virulent mycobacterium bovis strain. *The Journal of infectious diseases*, 208(11):1849–1858, December 2013. ISSN 1537-6613. doi: 10.1093/infdis/jit347. PMID: 23901081.

[567] Vijay A K Rathinam, Zhaozhao Jiang, Stephen N Waggoner, Shruti Sharma, Leah E Cole, Lisa Waggoner, Sivapriya Kailasan Vanaja, Brian G Monks, Sandhya Ganesan, Eicke Latz, Veit Hornung, Stefanie N Vogel, Eva Szomolanyi-Tsuda, and Katherine A Fitzgerald. The aim2 inflammasome is essential for host defense against cytosolic bacteria and dna viruses. *Nat Immunol*, 11(5):395–402, May 2010. doi: 10.1038/ni.1864.

[568] John-Demian Sauer, Chelsea E. Witte, Jason Zemansky, Bill Hanson, Peter Lauer, and Daniel A. Portnoy. Listeria monocytogenes triggers AIM2-Mediated pyroptosis upon infrequent bacteriolysis in the macrophage cytosol. *Cell Host & Microbe*, 7(5):412–419, May 2010. ISSN 1931-3128. doi: 10.1016/j.chom.2010.04.004. URL `http://www.sciencedirect.com/science/article/pii/S1931312810001101`.

[569] Jianghong Wu, Teresa Fernandes-Alnemri, and Emad S. Alnemri. Involvement of the AIM2, NLRC4, and NLRP3 inflammasomes in caspase-1 activation by listeria monocytogenes. *Journal of Clinical Immunology*, 30(5):693–702, September 2010. ISSN 0271-9142, 1573-2592. doi: 10.1007/s10875-010-9425-2. URL `http://link.springer.com/article/10.1007/s10875-010-9425-2`.

[570] Hiroyuki Saiga, Shoko Kitada, Yosuke Shimada, Naganori Kamiyama, Megumi Okuyama, Masahiko Makino, Masahiro Yamamoto, and Kiyoshi Takeda. Critical role of AIM2 in mycobacterium tuberculosis infection. *International Immunology*, 24(10):637–644, October 2012. ISSN 0953-8178, 1460-2377. doi: 10.1093/intimm/dxs062. URL `http://intimm.oxfordjournals.org/content/24/10/637`. PMID: 22695634.

[571] Gentao Liu, John S Yu, Gang Zeng, Dong Yin, Dong Xie, Keith L Black, and Han Ying. AIM-2: a novel tumor

antigen is expressed and presented by human glioma cells. *Journal of immunotherapy (Hagerstown, Md.: 1997)*, 27(3):220–226, June 2004. ISSN 1524-9557. PMID: 15076139.

[572] Andrea Ablasser, Franz Bauernfeind, Gunther Hartmann, Eicke Latz, Katherine A Fitzgerald, and Veit Hornung. RIG-I-dependent sensing of poly(dA:dT) through the induction of an RNA polymerase III-transcribed RNA intermediate. *Nature Immunology*, 10(10):1065–1072, October 2009. ISSN 1529-2916. doi: 10.1038/ni.1779.

[573] Xin Duan, Larissa Ponomareva, Sudhakar Veeranki, Ravichandran Panchanathan, Eric Dickerson, and Divaker Choubey. Differential roles for the interferon-inducible IFI16 and AIM2 innate immune sensors for cytosolic DNA in cellular senescence of human fibroblasts. *Molecular cancer research: MCR*, 9(5):589–602, May 2011. ISSN 1557-3125. doi: 10.1158/1541-7786.MCR-10-0565. PMID: 21471287 PMCID: PMC3096691.

[574] Sudhakar Veeranki and Divaker Choubey. Interferon-inducible p200-family protein IFI16, an innate immune sensor for cytosolic and nuclear double-stranded DNA: regulation of subcellular localization. *Molecular immunology*, 49 (4):567–571, January 2012. ISSN 1872-9142. doi: 10.1016/j.molimm.2011.11.004. PMID: 22137500 PMCID: PMC3249514.

[575] Kathryn M. Monroe, Zhiyuan Yang, Jeffrey R. Johnson, Xin Geng, Gilad Doitsh, Nevan J. Krogan, and Warner C. Greene. IFI16 DNA sensor is required for death of lymphoid CD4 t cells abortively infected with HIV. *Science*, 343(6169):428–432, January 2014. ISSN 0036-8075, 1095-9203. doi: 10.1126/science.1243640. URL http://www.sciencemag.org/content/343/6169/428. PMID: 24356113.

[576] Martin R. Jakobsen, Rasmus O. Bak, Annika Andersen, Randi K. Berg, Sren B. Jensen, Jin Tengchuan, Anders Laustsen, Kathrine Hansen, Lars stergaard, Katherine A. Fitzgerald, T. Sam Xiao, Jacob G. Mikkelsen, Trine H. Mogensen, and Sren R. Paludan. IFI16 senses DNA forms of the lentiviral replication cycle and controls HIV-1 replication. *Proceedings of the National Academy of Sciences*, page 201311669, October 2013. ISSN 0027-8424, 1091-6490. doi: 10.1073/pnas.1311669110. URL http://www.pnas.org/content/early/2013/10/23/1311669110. PMID: 24154727.

[577] Karen E Johnson, Leela Chikoti, and Bala Chandran. Hsv-1 infection induces activation and subsequent inhibition of the ifi16 and nlrp3 inflammasomes. *J Virol*, Feb 2013. doi: 10.1128/JVI.00082-13. URL http://dx.doi.org/10.1128/JVI.00082-13.

[578] Kathy Triantafilou, Dilan Eryilmazlar, and Martha Triantafilou. Herpes simplex virus 2-induced activation in vaginal cells involves toll-like receptors 2 and 9 and DNA sensors DAI and IFI16. *American journal of obstetrics and gynecology*, 210(2):122.e1–122.e10, February 2014. ISSN 1097-6868. doi: 10.1016/j.ajog.2013.09.034. PMID: 24080302.

[579] Grazia Rosaria Gariano, Valentina Dell'Oste, Matteo Bronzini, Deborah Gatti, Anna Luganini, Marco De Andrea, Giorgio Gribaudo, Marisa Gariglio, and Santo Landolfo. The intracellular DNA sensor IFI16 gene acts as restriction factor for human cytomegalovirus replication. *PLoS pathogens*, 8(1):e1002498, January 2012. ISSN 1553-7374. doi: 10.1371/journal.ppat.1002498. PMID: 22291595 PMCID: PMC3266931.

[580] Nagaraj Kerur, Mohanan Valiya Veettil, Neelam Sharma-Walia, Virginie Bottero, Sathish Sadagopan, Pushpalatha Otageri, and Bala Chandran. IFI16 acts as a nuclear pathogen sensor to induce the inflammasome in response to kaposi sarcoma-associated herpesvirus infection. *Cell host & microbe*, 9(5):363–375, May 2011. ISSN 1934-6069. doi: 10.1016/j.chom.2011.04.008. PMID: 21575908 PMCID: PMC3113467.

[581] Hideyuki Yanai, Tatsuma Ban, ZhiChao Wang, Myoung Kwon Choi, Takeshi Kawamura, Hideo Negishi, Makoto Nakasato, Yan Lu, Sho Hangai, Ryuji Koshiba, David Savitsky, Lorenza Ronfani, Shizuo Akira, Marco E Bianchi, Kenya Honda, Tomohiko Tamura, Tatsuhiko Kodama, and Tadatsugu Taniguchi. HMGB proteins function as universal sentinels for nucleic-acid-mediated innate immune responses. *Nature*, 462(7269):99–103, November 2009. ISSN 1476-4687. doi: 10.1038/nature08512.

[582] A. M. Krieg. CpG motifs in bacterial DNA trigger direct b-cell activation. *Nature*, 374:546–549, 1995. doi: 10.1038/374546a0.

[583] D. M. Klinman. Immunotherapeutic uses of CpG oligodeoxynucleotides. *Nature Rev. Immunol.*, 4:249–258, 2004. doi: 10.1038/nri1329.

[584] T. Haas. The DNA sugar backbone 2[prime] deoxyribose determines toll-like receptor 9 activation. *Immunity*, 28: 315–323, 2008. doi: 10.1016/j.immuni.2008.01.013.

[585] Pengyuan Yang, Huazhang An, Xingguang Liu, Mingyue Wen, Yuanyuan Zheng, Yaocheng Rui, and Xuetao Cao. The cytosolic nucleic acid sensor LRRFIP1 mediates the production of type i interferon via a [beta]-catenin-dependent pathway. *Nat Immunol*, 11(6):487–494, June 2010. ISSN 1529-2908. doi: 10.1038/ni.1876.

[586] S A Wilson, E C Brown, A J Kingsman, and S M Kingsman. TRIP: a novel double stranded RNA binding protein which interacts with the leucine rich repeat of flightless i. *Nucleic Acids Research*, 26(15):3460–3467, August 1998. ISSN 0305-1048.

[587] April R Suriano, Amy N Sanford, Nahmah Kim, Miae Oh, Sarah Kennedy, Mark J Henderson, Kelly Dietzmann, and Kathleen E Sullivan. GCF2/LRRFIP1 represses tumor necrosis factor alpha expression. *Molecular and Cellular Biology*, 25(20):9073–9081, October 2005. ISSN 0270-7306. doi: 10.1128/MCB.25.20.9073-9081.2005.

[588] Rachel Arakawa, Asen Bagashev, Li Song, Kelly Maurer, and Kathleen E Sullivan. Characterization of LRRFIP1. *Biochemistry and cell biology = Biochimie et biologie cellulaire*, 88(6):899–906, December 2010. ISSN 1208-6002. doi: 10.1139/O10-014. PMID: 21102652.

[589] Jennifer B Nguyen and Yorgo Modis. Crystal structure of the dimeric coiled-coil domain of the cytosolic nucleic acid sensor LRRFIP1. *Journal of structural biology*, 181(1):82–88, January 2013. ISSN 1095-8657. doi: 10.1016/j.jsb.2012.10.006. PMID: 23099021 PMCID: PMC3525766.

[590] Xavier Lahaye, Takeshi Satoh, Matteo Gentili, Silvia Cerboni, Ccile Conrad, Ilse Hurbain, Ahmed El Marjou, Christine Lacabaratz, Jean-Daniel Lelivre, and Nicolas Manel. The capsids of HIV-1 and HIV-2 determine immune detection of the viral cDNA by the innate sensor cGAS in dendritic cells. *Immunity*, 39(6):1132–1142, December 2013. ISSN 1097-4180. doi: 10.1016/j.immuni.2013.11.002. PMID: 24269171.

[591] Daxing Gao, Jiaxi Wu, You-Tong Wu, Fenghe Du, Chukwuemika Aroh, Nan Yan, Lijun Sun, and Zhijian J Chen. Cyclic GMP-AMP synthase is an innate immune sensor of HIV and other retroviruses. *Science (New York, N.Y.)*, 341(6148):903–906, August 2013. ISSN 1095-9203. doi: 10.1126/science.1240933. PMID: 23929945 PMCID: PMC3860819.

[592] Eric Lam, Saskia Stein, and Erik Falck-Pedersen. Adenovirus detection by the cGAS/STING/TBK1 DNA sensing cascade. *Journal of virology*, 88(2):974–981, January 2014. ISSN 1098-5514. doi: 10.1128/JVI.02702-13. PMID: 24198409 PMCID: PMC3911663.

[593] T. Vanden Berghe, N. Vanlangenakker, E. Parthoens, W. Deckers, M. Devos, N. Festjens, C. J. Guerin, U. T. Brunk, W. Declercq, and P. Vandenabeele. Necroptosis, necrosis and secondary necrosis converge on similar cellular disintegration features. *Cell Death & Differentiation*, 17(6):922–930, June 2010. ISSN 1350-9047. doi: 10.1038/cdd.2009.184. URL `http://www.nature.com/cdd/journal/v17/n6/abs/cdd2009184a.html`.

[594] G. Koopman, C. P. Reutelingsperger, G. A. Kuijten, R. M. Keehnen, S. T. Pals, and M. H. van Oers. Annexin v for flow cytometric detection of phosphatidylserine expression on b cells undergoing apoptosis. *Blood*, 84(5): 1415–1420, Sep 1994.

[595] Jonathan D. Edgeworth, Jo Spencer, Armelle Phalipon, George E. Griffin, and Philippe J. Sansonetti. Cytotoxicity and interleukin-1beta processing following shigella flexneri infection of human monocyte-derived dendritic cells. *Eur J Immunol*, 32(5):1464–1471, May 2002. doi: gt;3.0.CO;2-G. URL `http://dx.doi.org/gt;3.0.CO;2-G`.

[596] Ter Yong Tan and Justin Jang Hann Chu. Dengue virus-infected human monocytes trigger late activation of caspase-1, which mediates pro-inflammatory IL-1 secretion and pyroptosis. *The Journal of general virology*, 94(Pt 10):2215–2220, October 2013. ISSN 1465-2099. doi: 10.1099/vir.0.055277-0. PMID: 23884363.

[597] Gezahegn Gorfu, Kimberly M. Cirelli, Mariane B. Melo, Katrin Mayer-Barber, Devorah Crown, Beverly H. Koller, Seth Masters, Alan Sher, Stephen H. Leppla, Mahtab Moayeri, Jeroen P. J. Saeij, and Michael E. Grigg. Dual role

for inflammasome sensors NLRP1 and NLRP3 in murine resistance to toxoplasma gondii. *mBio*, 5(1):e01117–13, February 2014. ISSN , 2150-7511. doi: 10.1128/mBio.01117-13. URL http://mbio.asm.org/content/5/1/e01117-13. PMID: 24549849.

[598] Sarah E Ewald, Joseph Chavarria-Smith, and John C Boothroyd. NLRP1 is an inflammasome sensor for toxoplasma gondii. *Infection and immunity*, 82(1):460–468, January 2014. ISSN 1098-5522. doi: 10.1128/IAI.01170-13. PMID: 24218483 PMCID: PMC3911858.

[599] Changchun Fan. P2X4 promotes interleukin1 production in osteoarthritis via NLRP1. *Molecular Medicine Reports*, October 2013. ISSN 1791-2997, 1791-3004. doi: 10.3892/mmr.2013.1748. URL http://www.spandidos-publications.com/mmr/9/1/340.

[600] Jonathan L. Levinsohn, Zachary L. Newman, Kristina A. Hellmich, Rasem Fattah, Matthew A. Getz, Shihui Liu, Inka Sastalla, Stephen H. Leppla, and Mahtab Moayeri. Anthrax lethal factor cleavage of nlrp1 is required for activation of the inflammasome. *PLoS Pathog*, 8(3):e1002638, March 2012. doi: 10.1371/journal.ppat.1002638. URL http://dx.doi.org/10.1371/journal.ppat.1002638.

[601] Yue Zhao, Jieling Yang, Jianjin Shi, Yi-Nan Gong, Qiuhe Lu, Hao Xu, Liping Liu, and Feng Shao. The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus. *Nature*, 477(7366):596–600, September 2011. ISSN 1476-4687. doi: 10.1038/nature10510. PMID: 21918512.

[602] Edward A Miao, Dat P Mao, Natalya Yudkovsky, Richard Bonneau, Cynthia G Lorang, Sarah E Warren, Irina A Leaf, and Alan Aderem. Innate immune detection of the type III secretion apparatus through the NLRC4 inflammasome. *Proceedings of the National Academy of Sciences of the United States of America*, 107(7):3076–3080, February 2010. ISSN 1091-6490. doi: 10.1073/pnas.0913087107. PMID: 20133635 PMCID: PMC2840275.

[603] Susan L. Fink, Tessa Bergsbaken, and Brad T. Cookson. Anthrax lethal toxin and salmonella elicit the common cell death pathway of caspase-1-dependent pyroptosis via distinct mechanisms. *Proc Natl Acad Sci U S A*, 105(11): 4312–4317, Mar 2008. doi: 10.1073/pnas.0707370105. URL http://dx.doi.org/10.1073/pnas.0707370105.

[604] Laurence Feldmeyer, Martin Keller, Gisela Niklaus, Daniel Hohl, Sabine Werner, and Hans-Dietmar Beer. The inflammasome mediates uvb-induced activation and secretion of interleukin-1beta by keratinocytes. *Curr Biol*, 17 (13):1140–1145, Jul 2007. doi: 10.1016/j.cub.2007.05.074. URL http://dx.doi.org/10.1016/j.cub.2007.05.074.

[605] Claudia Giampietri, Donatella Starace, Simonetta Petrungaro, Antonio Filippini, and Elio Ziparo. Necroptosis: Molecular signalling and translational implications. *International Journal of Cell Biology*, 2014, January 2014. ISSN 1687-8876. doi: 10.1155/2014/490275. URL http://www.hindawi.com/journals/ijcb/2014/490275/abs/.

[606] N Holler, R Zaru, O Micheau, M Thome, A Attinger, S Valitutti, J L Bodmer, P Schneider, B Seed, and J Tschopp. Fas triggers an alternative, caspase-8-independent cell death pathway using the kinase RIP as effector molecule. *Nature immunology*, 1(6):489–495, December 2000. ISSN 1529-2908. doi: 10.1038/82732. PMID: 11101870.

[607] N. Vanlangenakker, M. J. M. Bertrand, P. Bogaert, P. Vandenabeele, and T. Vanden Berghe. TNF-induced necroptosis in l929 cells is tightly regulated by multiple TNFR1 complex i and II members. *Cell Death & Disease*, 2(11):e230, November 2011. doi: 10.1038/cddis.2011.111. URL http://www.nature.com/cddis/journal/v2/n11/abs/cddis2011111a.html.

[608] Irene L. Chen, Jennifer S. Tsau, Jeffery D. Molkentin, Masaaki Komatsu, and Stephen M. Hedrick. Mechanisms of necroptosis in t cells. *The Journal of Experimental Medicine*, 208(4):633–641, April 2011. ISSN 0022-1007, 1540-9538. doi: 10.1084/jem.20110251. URL http://jem.rupress.org/content/208/4/633. PMID: 21402742.

[609] K.-J. Song, Y. S. Jang, Y. A. Lee, K. A. Kim, S. K. Lee, and M. H. Shin. Reactive oxygen species-dependent necroptosis in jurkat t cells induced by pathogenic free-living naegleria fowleri. *Parasite Immunology*, 33(7):390–400, July 2011. ISSN 1365-3024. doi: 10.1111/j.1365-3024.2011.01297.x. URL http://onlinelibrary.wiley.com/doi/10.1111/j.1365-3024.2011.01297.x/abstract.

[610] Jin-Young Han, Derek D Sloan, Martine Aubert, Sara A Miller, Chung H Dang, and Keith R Jerome. Apoptosis

and antigen receptor function in t and b cells following exposure to herpes simplex virus. *Virology*, 359(2):253–263, Mar 2007. doi: 10.1016/j.virol.2006.09.038. URL http://dx.doi.org/10.1016/j.virol.2006.09.038.

[611] Diane B Re, Virginia Le Verche, Changhao Yu, Mackenzie W Amoroso, Kristin A Politi, Sudarshan Phani, Burcin Ikiz, Lucas Hoffmann, Martijn Koolen, Tetsuya Nagata, Dimitra Papadimitriou, Peter Nagy, Hiroshi Mitsumoto, Shingo Kariya, Hynek Wichterle, Christopher E Henderson, and Serge Przedborski. Necroptosis drives motor neuron death in models of both sporadic and familial ALS. *Neuron*, 81(5):1001–1008, March 2014. ISSN 1097-4199. doi: 10.1016/j.neuron.2014.01.011. PMID: 24508385 PMCID: PMC3951532.

[612] Ye-Fa Zhang, Wei He, Cheng Zhang, Xiao-Jing Liu, Yan Lu, Hua Wang, Zhi-Hui Zhang, Xi Chen, and De-Xiang Xu. Role of receptor interacting protein (RIP)1 on apoptosis-inducing factor-mediated necroptosis during acetaminophen-evoked acute liver failure in mice. *Toxicology Letters*, 225(3):445–453, March 2014. ISSN 0378-4274. doi: 10.1016/j.toxlet.2014.01.005. URL http://www.sciencedirect.com/science/article/pii/S0378427414000150.

[613] Federica Rizzi, Valeria Naponelli, Alessandro Silva, Alice Modernelli, Ileana Ramazzina, Martina Bonacini, Saverio Tardito, Rita Gatti, Jacopo Uggeri, and Saverio Bettuzzi. Polyphenon e(r), a standardized green tea extract, induces endoplasmic reticulum stress, leading to death of immortalized PNT1a cells by anoikis and tumorigenic PC3 by necroptosis. *Carcinogenesis*, January 2014. ISSN 1460-2180. doi: 10.1093/carcin/bgt481. PMID: 24343359.

[614] Shizuka Koshinuma, Masami Miyamae, Kazuhiro Kaneda, Junichiro Kotani, and Vincent M Figueredo. Combination of necroptosis and apoptosis inhibition enhances cardioprotection against myocardial ischemia-reperfusion injury. *Journal of anesthesia*, October 2013. ISSN 1438-8359. doi: 10.1007/s00540-013-1716-3. PMID: 24113863.

[615] A. Lau, S. Wang, J. Jiang, A. Haig, A. Pavlosky, A. Linkermann, Z.-X. Zhang, and A. M. Jevnikar. RIPK3-Mediated necroptosis promotes donor kidney inflammatory injury and reduces allograft survival. *American Journal of Transplantation*, 13(11):2805–2818, November 2013. ISSN 1600-6143. doi: 10.1111/ajt.12447. URL http://onlinelibrary.wiley.com/doi/10.1111/ajt.12447/abstract.

[616] H Ankel, D F Westra, S Welling-Wester, and P Lebon. Induction of interferon-alpha by glycoprotein d of herpes simplex virus: a possible role of chemokine receptors. *Virology*, 251(2):317–326, November 1998. ISSN 0042-6822. doi: 10.1006/viro.1998.9432.

[617] Gabriele Pollara, David R Katz, and Benjamin M Chain. The host response to herpes simplex virus infection. *Current opinion in infectious diseases*, 17(3):199–203, June 2004. ISSN 0951-7375. PMID: 15166821.

[618] Simon B. Rasmussen, Louise N. Srensen, Lene Malmgaard, Nina Ank, Joel D. Baines, Zhijian J. Chen, and Sren R. Paludan. Type i interferon production during herpes simplex virus infection is controlled by Cell-Type-Specific viral recognition through Toll-Like receptor 9, the mitochondrial antiviral signaling protein pathway, and novel recognition systems. *Journal of Virology*, 81(24):13315–13324, December 2007. ISSN 0022-538X. doi: 10.1128/JVI.01167-07.

[619] Jennifer Lund, Ayuko Sato, Shizuo Akira, Ruslan Medzhitov, and Akiko Iwasaki. Toll-like receptor 9mediated recognition of herpes simplex virus-2 by plasmacytoid dendritic cells. *The Journal of Experimental Medicine*, 198 (3):513 –520, 2003. doi: 10.1084/jem.20030162.

[620] Anne Krug, Gary D. Luker, Winfried Barchet, David A. Leib, Shizuo Akira, and Marco Colonna. Herpes simplex virus type 1 activates murine natural interferon-producing cells through toll-like receptor 9. *Blood*, 103(4):1433–1437, February 2004. doi: 10.1182/blood-2003-08-2674.

[621] Carlos J. Montoya, Hyun-Bae Jie, Lena Al-Harthi, Candice Mulder, Pablo J. Patio, Mara T. Rugeles, Arthur M. Krieg, Alan L. Landay, and S. Brian Wilson. Activation of plasmacytoid dendritic cells with TLR9 agonists initiates invariant NKT cell-mediated cross-talk with myeloid dendritic cells. *The Journal of Immunology*, 177(2):1028–1039, July 2006. ISSN 0022-1767, 1550-6606. URL http://www.jimmunol.org/content/177/2/1028. PMID: 16818759.

[622] Yu-Hsin Chiu, John B Macmillan, and Zhijian J Chen. RNA polymerase III detects cytosolic DNA and induces type i interferons through the RIG-I pathway. *Cell*, 138(3):576–591, August 2009. ISSN 1097-4172. doi: 10.1016/j.cell.2009.06.015.

[623] Zhiqiang Zhang, Taeil Kim, Musheng Bao, Valeria Facchinetti, Sung Yun Jung, Amir Ali Ghaffari, Jun Qin, Genhong Cheng, and Yong-Jun Liu. DDX1, DDX21, and DHX36 helicases form a complex with the adaptor molecule TRIF to sense dsRNA in dendritic cells. *Immunity*, 34(6):866–878, June 2011. ISSN 1097-4180. doi: 10.1016/j.immuni.2011.03.027. PMID: 21703541 PMCID: PMC3652560.

[624] Jin-Young Han, Sara A Miller, Teresa M Wolfe, Hoda Pourhassan, and Keith R Jerome. Cell type-specific induction and inhibition of apoptosis by herpes simplex virus type 2 icp10. *J Virol*, 83(6):2765–2769, Mar 2009. doi: 10.1128/JVI.02088-08. URL http://dx.doi.org/10.1128/JVI.02088-08.

[625] Martine Aubert, Lisa E Pomeranz, and John A Blaho. Herpes simplex virus blocks apoptosis by precluding mitochondrial cytochrome c release independent of caspase activation in infected human epithelial cells. *Apoptosis*, 12 (1):19–35, Jan 2007. doi: 10.1007/s10495-006-0330-3. URL http://dx.doi.org/10.1007/s10495-006-0330-3.

[626] Sarah L Grady, Jesse Hwang, Livia Vastag, Joshua D Rabinowitz, and Thomas Shenk. Herpes simplex virus 1 infection activates poly(adp-ribose) polymerase and triggers the degradation of poly(adp-ribose) glycohydrolase. *J Virol*, 86(15):8259–8268, Aug 2012. doi: 10.1128/JVI.00495-12. URL http://dx.doi.org/10.1128/JVI.00495-12.

[627] Simon B Rasmussen, Kristy A Horan, Christian K Holm, Amanda J Stranks, Thomas C Mettenleiter, A. Katharina Simon, Sren B Jensen, Frazer J Rixon, Bin He, and Sren R Paludan. Activation of autophagy by ?-herpesviruses in myeloid cells is mediated by cytoplasmic viral dna through a mechanism dependent on stimulator of ifn genes. *J Immunol*, 187(10):5268–5276, Nov 2011. doi: 10.4049/jimmunol.1100949. URL http://dx.doi.org/10.4049/jimmunol.1100949.

[628] W R Sacks, C C Greene, D P Aschman, and P A Schaffer. Herpes simplex virus type 1 ICP27 is an essential regulatory protein. *Journal of Virology*, 55(3):796–805, September 1985. ISSN 0022-538X. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC255064/. PMID: 2991596 PMCID: PMC255064.

[629] G. Zachos, M. Koffa, C. M. Preston, J. B. Clements, and J. Conner. Herpes simplex virus type 1 blocks the apoptotic host cell defense mechanisms that target bcl-2 and manipulates activation of p38 mitogen-activated protein kinase to improve viral replication. *J Virol*, 75(6):2710–2728, Mar 2001. doi: 10.1128/JVI.75.6.2710-2728.2001. URL http://dx.doi.org/10.1128/JVI.75.6.2710-2728.2001.

[630] Danna Hargett, Tim McLean, and Steven L Bachenheimer. Herpes simplex virus icp27 activation of stress kinases jnk and p38. *J Virol*, 79(13):8348–8360, Jul 2005. doi: 10.1128/JVI.79.13.8348-8360.2005. URL http://dx.doi.org/10.1128/JVI.79.13.8348-8360.2005.

[631] R. Leopardi, C. Van Sant, and B. Roizman. The herpes simplex virus 1 protein kinase us3 is required for protection from apoptosis induced by the virus. *Proc Natl Acad Sci U S A*, 94(15):7891–7896, Jul 1997.

[632] A. E. Reynolds, B. J. Ryckman, J. D. Baines, Y. Zhou, L. Liang, and R. J. Roller. U(l)31 and u(l)34 proteins of herpes simplex virus type 1 form a complex that accumulates at the nuclear rim and is required for envelopment of nucleocapsids. *J Virol*, 75(18):8803–8817, Sep 2001.

[633] T. D. Chung, J. P. Wymer, C. C. Smith, M. Kulka, and L. Aurelian. Protein kinase activity associated with the large subunit of herpes simplex virus type 2 ribonucleotide reductase (icp10). *J Virol*, 63(8):3389–3398, Aug 1989.

[634] C. C. Smith, J. Nelson, L. Aurelian, M. Gober, and B. B. Goswami. Ras-gap binding and phosphorylation by herpes simplex virus type 2 rr1 pk (icp10) and activation of the ras/mek/mapk mitogenic pathway are required for timely onset of virus growth. *J Virol*, 74(22):10417–10429, Nov 2000.

[635] C. C. Smith, T. Peng, M. Kulka, and L. Aurelian. The pk domain of the large subunit of herpes simplex virus type 2 ribonucleotide reductase (icp10) is required for immediate-early gene expression and virus growth. *J Virol*, 72 (11):9131–9141, Nov 1998.

[636] L. Aurelian and C. C. Smith. Herpes simplex virus type 2 growth and latency reactivation by cocultivation are inhibited with antisense oligonucleotides complementary to the translation initiation site of the large subunit of ribonucleotide reductase (rr1). *Antisense Nucleic Acid Drug Dev*, 10(2):77–85, Apr 2000.

[637] Michael D Gober, Samantha Q Wales, J. Colin Hunter, Bhuvnesh K Sharma, and Laure Aurelian. Stress up-regulates neuronal expression of the herpes simplex virus type 2 large subunit of ribonucleotide reductase (r1; icp10) by activating activator protein 1. *J Neurovirol*, 11(4):329–336, Aug 2005. doi: 10.1080/13550280591002423. URL http://dx.doi.org/10.1080/13550280591002423.

[638] Samantha Q Wales, Baiquan Li, Jennifer M Laing, and Laure Aurelian. The herpes simplex virus type 2 gene icp10pk protects from apoptosis caused by nerve growth factor deprivation through inhibition of caspase-3 activation and xiap up-regulation. *J Neurochem*, 103(1):365–379, Oct 2007. doi: 10.1111/j.1471-4159.2007.04745.x. URL http://dx.doi.org/10.1111/j.1471-4159.2007.04745.x.

[639] S. Q. Wales, J. M. Laing, L. Chen, and L. Aurelian. Icp10pk inhibits calpain-dependent release of apoptosis-inducing factor and programmed cell death in response to the toxin mpp+. *Gene Ther*, 15(20):1397–1409, Oct 2008. doi: 10.1038/gt.2008.88. URL http://dx.doi.org/10.1038/gt.2008.88.

[640] Laure Aurelian, Jennifer M Laing, and Ki Seok Lee. H11/hspb8 and its herpes simplex virus type 2 homologue icp10pk share functions that regulate cell life/death decisions and human disease. *Autoimmune Dis*, 2012:395329, 2012. doi: 10.1155/2012/395329. URL http://dx.doi.org/10.1155/2012/395329.

[641] Florent Dufour, A. Marie-Jose Sasseville, Stphane Chabaud, Bernard Massie, Richard M Siegel, and Yves Langelier. The ribonucleotide reductase r1 subunits of herpes simplex virus types 1 and 2 protect cells against tnf?- and fasl-induced apoptosis by interacting with caspase-8. *Apoptosis*, 16(3):256–271, Mar 2011. doi: 10.1007/s10495-010-0560-2. URL http://dx.doi.org/10.1007/s10495-010-0560-2.

[642] J. C. Hunter, C. C. Smith, D. Bose, M. Kulka, R. Broderick, and L. Aurelian. Intracellular internalization and signaling pathways triggered by the large subunit of hsv-2 ribonucleotide reductase (icp10). *Virology*, 210(2): 345–360, Jul 1995. doi: 10.1006/viro.1995.1351. URL http://dx.doi.org/10.1006/viro.1995.1351.

[643] D. Perkins, E. F R Pereira, and L. Aurelian. The herpes simplex virus type 2 r1 protein kinase (icp10 pk) functions as a dominant regulator of apoptosis in hippocampal neurons involving activation of the erk survival pathway and upregulation of the antiapoptotic protein bag-1. *J Virol*, 77(2):1292–1305, Jan 2003.

[644] Jennifer M Laing, Erin K Golembewski, Samantha Q Wales, Juan Liu, M. Samir Jafri, Paul J Yarowsky, and Laure Aurelian. Growth-compromised hsv-2 vector delta rr protects from n-methyl-d-aspartate-induced neuronal degeneration through redundant activation of the mek/erk and pi3-k/akt survival pathways, either one of which overrides apoptotic cascades. *J Neurosci Res*, 86(2):378–391, Feb 2008. doi: 10.1002/jnr.21486. URL http://dx.doi.org/10.1002/jnr.21486.

[645] Jennifer M Laing, Cynthia C Smith, and Laure Aurelian. Multi-targeted neuroprotection by the hsv-2 gene icp10pk includes robust bystander activity through pi3-k/akt and/or mek/erk-dependent neuronal release of vascular endothelial growth factor and fractalkine. *J Neurochem*, 112(3):662–676, Feb 2010. doi: 10.1111/j.1471-4159.2009.06475.x. URL http://dx.doi.org/10.1111/j.1471-4159.2009.06475.x.

[646] G. Zhou, V. Galvan, G. Campadelli-Fiume, and B. Roizman. Glycoprotein d or j delivered in trans blocks apoptosis in sk-n-sh cells induced by a herpes simplex virus 1 mutant lacking intact genes expressing both glycoproteins. *J Virol*, 74(24):11782–11791, Dec 2000.

[647] G. Zhou and B. Roizman. The domains of glycoprotein d required to block apoptosis depend on whether glycoprotein d is present in the virions carrying herpes simplex virus 1 genome lacking the gene encoding the glycoprotein. *J Virol*, 75(13):6166–6172, Jul 2001. doi: 10.1128/JVI.75.13.6166-6172.2001. URL http://dx.doi.org/10.1128/JVI.75.13.6166-6172.2001.

[648] M. Antonietta Medici, M. Teresa Sciortino, Donata Perri, Carla Amici, Elisa Avitabile, Marco Ciotti, Emanuela Balestrieri, Enrico De Smaele, Guido Franzoso, and Antonio Mastino. Protection by herpes simplex virus glycoprotein d against fas-mediated apoptosis: role of nuclear factor kappab. *J Biol Chem*, 278(38):36059–36067, Sep 2003. doi: 10.1074/jbc.M306198200. URL http://dx.doi.org/10.1074/jbc.M306198200.

[649] Xianzhi Jiang, Aziz Alami Chentoufi, Chinhui Hsiang, Dale Carpenter, Nelson Osorio, Lbachir BenMohamed, Nigel W Fraser, Clinton Jones, and Steven L Wechsler. The herpes simplex virus type 1 latency-associated transcript

can protect neuron-derived c1300 and neuro2a cells from granzyme b-induced apoptosis and cd8 t-cell killing. *J Virol*, 85(5):2325–2332, Mar 2011. doi: 10.1128/JVI.01791-10. URL `http://dx.doi.org/10.1128/JVI.01791-10`.

[650] James Cb Li, Kin-Yi Au, Jun-Wei Fang, Howard Ch Yim, Kin-Hung Chow, Pak-Leung Ho, and Allan Sy Lau. HIV-1 trans-activator protein dysregulates IFN- signaling and contributes to the suppression of autophagy induction. *AIDS (London, England)*, November 2010. ISSN 1473-5571. doi: 10.1097/QAD.0b013e328340fd61.

[651] A. Patel, J. Hanson, T. I. McLean, J. Olgiate, M. Hilton, W. E. Miller, and S. L. Bachenheimer. Herpes simplex type 1 induction of persistent nf-kappa b nuclear translocation increases the efficiency of virus replication. *Virology*, 247(2):212–222, Aug 1998.

[652] Margot L Goodkin, Adrian T Ting, and John A Blaho. Nf-kappab is required for apoptosis prevention during herpes simplex virus type 1 infection. *J Virol*, 77(13):7261–7280, Jul 2003.

[653] D. Gregory, D. Hargett, D. Holmes, E. Money, and S. L. Bachenheimer. Efficient replication by herpes simplex virus type 1 involves activation of the ikappab kinase-ikappab-p65 pathway. *J Virol*, 78(24):13582–13590, Dec 2004. doi: 10.1128/JVI.78.24.13582-13590.2004. URL `http://dx.doi.org/10.1128/JVI.78.24.13582-13590.2004`.

[654] Jamie C Yedowitz and John A Blaho. Herpes simplex virus 2 modulates apoptosis and stimulates nf-kappab nuclear translocation during infection in human epithelial hep-2 cells. *Virology*, 342(2):297–310, Nov 2005. doi: 10.1016/j.virol.2005.07.036. URL `http://dx.doi.org/10.1016/j.virol.2005.07.036`.

[655] M. Krzyzowska, A. Shestakov, K. Eriksson, and F. Chiodi. Role of fas/fasl in regulation of inflammation in vaginal tissue during hsv-2 infection. *Cell Death Dis*, 2:e132, 2011. doi: 10.1038/cddis.2011.14. URL `http://dx.doi.org/10.1038/cddis.2011.14`.

[656] Luca Benetti and Bernard Roizman. Protein kinase b/akt is present in activated form throughout the entire replicative cycle of deltau(s)3 mutant virus but only at early times after infection with wild-type herpes simplex virus 1. *J Virol*, 80(7):3341–3348, Apr 2006. doi: 10.1128/JVI.80.7.3341-3348.2006. URL `http://dx.doi.org/10.1128/JVI.80.7.3341-3348.2006`.

[657] Mei-Ju Hsu, Ching-Yi Wu, Hsiao-Han Chiang, Yu-Lin Lai, and Shan-Ling Hung. Pi3k/akt signaling mediated apoptosis blockage and viral gene expression in oral epithelial cells during herpes simplex virus infection. *Virus Res*, 153(1):36–43, Oct 2010. doi: 10.1016/j.virusres.2010.07.002. URL `http://dx.doi.org/10.1016/j.virusres.2010.07.002`.

[658] Melany J Wagner and James R Smiley. Herpes simplex virus requires vp11/12 to activate src family kinase-phosphoinositide 3-kinase-akt signaling. *J Virol*, 85(6):2803–2812, Mar 2011. doi: 10.1128/JVI.01877-10. URL `http://dx.doi.org/10.1128/JVI.01877-10`.

[659] K A Jones and B M Peterlin. Control of RNA initiation and elongation at the HIV-1 promoter. *Annual review of biochemistry*, 63:717–743, 1994. ISSN 0066-4154. doi: 10.1146/annurev.bi.63.070194.003441. PMID: 7979253.

[660] X Lu, T M Welsh, and B M Peterlin. The human immunodeficiency virus type 1 long terminal repeat specifies two different transcription complexes, only one of which is regulated by tat. *Journal of virology*, 67(4):1752–1760, April 1993. ISSN 0022-538X. PMID: 8445708 PMCID: PMC240213.

[661] H S Olsen and C A Rosen. Contribution of the TATA motif to tat-mediated transcriptional activation of human immunodeficiency virus gene expression. *Journal of virology*, 66(9):5594–5597, September 1992. ISSN 0022-538X. PMID: 1501293 PMCID: PMC289121.

[662] B Berkhout and K T Jeang. Functional roles for the TATA promoter and enhancers in basal and tat-induced expression of the human immunodeficiency virus type 1 long terminal repeat. *Journal of virology*, 66(1):139–149, January 1992. ISSN 0022-538X. PMID: 1727476 PMCID: PMC238269.

[663] Ben Berkhout, Robert H. Silverman, and Kuan-Teh Jeang. Tat trans-activates the human immunodeficiency virus through a nascent RNA target. *Cell*, 59(2):273–282, October 1989. ISSN 0092-8674. doi: 10.1016/0092-8674(89)90289-4. URL `http://www.sciencedirect.com/science/article/pii/0092867489902894`.

[664] C H Herrmann and A P Rice. Lentivirus tat proteins specifically associate with a cellular protein kinase, TAK, that hyperphosphorylates the carboxyl-terminal domain of the large subunit of RNA polymerase II: candidate for a tat cofactor. *Journal of virology*, 69(3):1612–1620, March 1995. ISSN 0022-538X. PMID: 7853496 PMCID: PMC188757.

[665] H Kato, H Sumimoto, P Pognonec, C H Chen, C A Rosen, and R G Roeder. HIV-1 tat acts as a processivity factor in vitro in conjunction with cellular elongation factors. *Genes & development*, 6(4):655–666, April 1992. ISSN 0890-9369. PMID: 1559613.

[666] F Kashanchi, G Piras, M F Radonovich, J F Duvall, A Fattaey, C M Chiang, R G Roeder, and J N Brady. Direct interaction of human TFIID with the HIV-1 transactivator tat. *Nature*, 367(6460):295–299, January 1994. ISSN 0028-0836. doi: 10.1038/367295a0. PMID: 8121496.

[667] P Veschambre, A Roisin, and P Jalinot. Biochemical and functional interaction of the human immunodeficiency virus type 1 tat transactivator with the general transcription factor TFIIB. *The Journal of general virology*, 78 (Pt 9):2235–2245, September 1997. ISSN 0022-1317. PMID: 9292011.

[668] C A Parada and R G Roeder. Enhanced processivity of RNA polymerase II triggered by tat-induced phosphorylation of its carboxy-terminal domain. *Nature*, 384(6607):375–378, November 1996. ISSN 0028-0836. doi: 10.1038/384375a0. PMID: 8934526.

[669] F Wu-Baer, D Sigman, and R B Gaynor. Specific binding of RNA polymerase II to the human immunodeficiency virus trans-activating region RNA is regulated by cellular cofactors and tat. *Proceedings of the National Academy of Sciences of the United States of America*, 92(16):7153–7157, August 1995. ISSN 0027-8424. PMID: 7638159 PMCID: PMC41297.

[670] Tamal Raha, S W Grace Cheng, and Michael R Green. HIV-1 tat stimulates transcription complex assembly through recruitment of TBP in the absence of TAFs. *PLoS biology*, 3(2):e44, February 2005. ISSN 1545-7885. doi: 10.1371/journal.pbio.0030044. PMID: 15719058 PMCID: PMC546330.

[671] Pratima Rawat and Debashis Mitra. Cellular heat shock factor 1 positively regulates human immunodeficiency virus-1 gene expression and replication by two distinct pathways. *Nucleic acids research*, 39(14):5879–5892, August 2011. ISSN 1362-4962. doi: 10.1093/nar/gkr198. PMID: 21459854 PMCID: PMC3152347.

[672] Suryaram Gummuluru and Michael Emerman. Cell cycle- and vpr-mediated regulation of human immunodeficiency virus type 1 expression in primary and transformed t-cell lines. *Journal of Virology*, 73(7):5422–5430, July 1999. ISSN 0022-538X, 1098-5514. URL http://jvi.asm.org/content/73/7/5422. PMID: 10364289.

[673] Wei Chun Goh, Mark E. Rogel, C. Matthew Kinsey, Scott F. Michael, Patricia N. Fultz, Martin A. Nowak, Beatrice H. Hahn, and Michael Emerman. HIV-1 vpr increases viral expression by manipulation of the cell cycle: A mechanism for selection of vpr in vivo. *Nature Medicine*, 4(1):65–71, January 1998. doi: 10.1038/nm0198-065. URL http://www.nature.com.libproxy.ucl.ac.uk/nm/journal/v4/n1/abs/nm0198-065.html.

[674] R Vanitharani, S Mahalingam, Y Rafaeli, S P Singh, A Srinivasan, D B Weiner, and V Ayyavoo. HIV-1 vpr transactivates LTR-directed expression through sequences present within -278 to -176 and increases virus replication in vitro. *Virology*, 289(2):334–342, October 2001. ISSN 0042-6822. doi: 10.1006/viro.2001.1153. PMID: 11689055.

[675] H S Mancebo, G Lee, J Flygare, J Tomassini, P Luu, Y Zhu, J Peng, C Blau, D Hazuda, D Price, and O Flores. P-TEFb kinase is required for HIV tat transcriptional activation in vivo and in vitro. *Genes & development*, 11(20): 2633–2644, October 1997. ISSN 0890-9369. PMID: 9334326 PMCID: PMC316604.

[676] B Matija Peterlin and David H Price. Controlling the elongation phase of transcription with p-TEFb. *Molecular cell*, 23(3):297–305, August 2006. ISSN 1097-2765. doi: 10.1016/j.molcel.2006.06.014. PMID: 16885020.

[677] S M Carty, A C Goldstrohm, C Su, M A Garcia-Blanco, and A L Greenleaf. Protein-interaction modules that organize nuclear function: FF domains of CA150 bind the phosphoCTD of RNA polymerase II. *Proceedings of the National Academy of Sciences of the United States of America*, 97(16):9015–9020, August 2000. ISSN 0027-8424. doi: 10.1073/pnas.160266597. PMID: 10908677 PMCID: PMC16813.

[678] Mayte Coiras, Marta Montes, Immaculada Montanuy, Mara R. Lpez-Huertas, Elena Mateos, Caroline Le Sommer, Mariano A. Garcia-Blanco, Cristina Hernndez-Munain, Jos Alcam, and Carlos Su. Transcription elongation regulator 1 (TCERG1) regulates competent RNA polymerase II-mediated elongation of HIV-1 transcription and facilitates efficient viral replication. *Retrovirology*, 10(1):124, October 2013. ISSN 1742-4690. doi: 10.1186/1742-4690-10-124. URL http://www.retrovirology.com/content/10/1/124/abstract. PMID: 24165037.

[679] A. el Kharroubi and E. Verdin. Protein-DNA interactions within DNase i-hypersensitive sites located downstream of the HIV-1 promoter. *Journal of Biological Chemistry*, 269(31):19916–19924, August 1994. ISSN 0021-9258, 1083-351X. URL http://www.jbc.org/content/269/31/19916. PMID: 8051074.

[680] C. Van Lint, C. A. Amella, S. Emiliani, M. John, T. Jie, and E. Verdin. Transcription factor binding sites downstream of the human immunodeficiency virus type 1 transcription start site are important for virus infectivity. *Journal of Virology*, 71(8):6113–6127, August 1997. ISSN 0022-538X, 1098-5514. URL http://jvi.asm.org/content/71/8/6113. PMID: 9223506.

[681] A. Peeters, P. F. Lambert, and N. J. Deacon. A fourth sp1 site in the human immunodeficiency virus type 1 long terminal repeat is essential for negative-sense transcription. *Journal of Virology*, 70(10):6665–6672, October 1996. ISSN 0022-538X, 1098-5514. URL http://jvi.asm.org/content/70/10/6665. PMID: 8794302.

[682] D Harrich, J Garcia, F Wu, R Mitsuyasu, J Gonazalez, and R Gaynor. Role of SP1-binding domains in in vivo transcriptional regulation of the human immunodeficiency virus type 1 long terminal repeat. *Journal of Virology*, 63 (6):2585–2591, June 1989. ISSN 0022-538X. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC250732/. PMID: 2657100 PMCID: PMC250732.

[683] Lihua Shi, Juan C Perin, Jeremy Leipzig, Zhe Zhang, and Kathleen E Sullivan. Genome-wide analysis of interferon regulatory factor i binding in primary human monocytes. *Gene*, 487(1):21–28, November 2011. ISSN 1879-0038. doi: 10.1016/j.gene.2011.07.004. PMID: 21803131 PMCID: PMC3167955.

[684] M F Rabbi, M Saifuddin, D S Gu, M F Kagnoff, and K A Roebuck. U5 region of the human immunodeficiency virus type 1 long terminal repeat contains TRE-like cAMP-responsive elements that bind both AP-1 and CREB/ATF proteins. *Virology*, 233(1):235–245, June 1997. ISSN 0042-6822. doi: 10.1006/viro.1997.8602. PMID: 9201233.

[685] M O Hottiger and G J Nabel. Interaction of human immunodeficiency virus type 1 tat with the transcriptional coactivators p300 and CREB binding protein. *Journal of virology*, 72(10):8252–8256, October 1998. ISSN 0022-538X. PMID: 9733868 PMCID: PMC110181.

[686] G Marzio, M Tyagi, M I Gutierrez, and M Giacca. HIV-1 tat transactivator recruits p300 and CREB-binding protein histone acetyltransferases to the viral promoter. *Proceedings of the National Academy of Sciences of the United States of America*, 95(23):13519–13524, November 1998. ISSN 0027-8424. PMID: 9811832 PMCID: PMC24851.

[687] Eileen S. Lee, Huiyu Zhou, and Andrew J. Henderson. Endothelial cells enhance human immunodeficiency virus type 1 replication in macrophages through a C/EBP-Dependent mechanism. *Journal of Virology*, 75(20):9703–9712, October 2001. ISSN 0022-538X, 1098-5514. doi: 10.1128/JVI.75.20.9703-9712.2001. URL http://jvi.asm.org/content/75/20/9703. PMID: 11559803.

[688] F. Canonne-Hergaux, D. Aunis, and E. Schaeffer. Interactions of the transcription factor AP-1 with the long terminal repeat of different human immunodeficiency virus type 1 strains in jurkat, glial, and neuronal cells. *Journal of Virology*, 69(11):6634–6642, November 1995. ISSN 0022-538X, 1098-5514. URL http://jvi.asm.org/content/69/11/6634. PMID: 7474072.

[689] Gary Nabel and David Baltimore. An inducible transcription factor activates expression of human immunodeficiency virus in t cells. *Nature*, 326(6114):711–713, April 1987. doi: 10.1038/326711a0. URL http://www.nature.com.libproxy.ucl.ac.uk/nature/journal/v326/n6114/abs/326711a0.html.

[690] Massimo Mallardo, Emilia Dragonetti, Francesca Baldassarre, Concetta Ambrosino, Giuseppe Scala, and Ileana Quinto. An NF-B site in the 5-untranslated leader region of the human immunodeficiency virus type 1 enhances the viral expression in response to NF-B-activating stimuli. *Journal of Biological Chemistry*, 271(34):20820–20827, August 1996. ISSN 0021-9258, 1083-351X. URL http://www.jbc.org/content/271/34/20820. PMID: 8702837.

[691] Shigemi Kinoshita, Lishan Su, Masahiko Amano, Luika A Timmerman, Hideto Kaneshima, and Garry P Nolan. The t cell activation factor NF-ATc positively regulates HIV-1 replication and gene expression in t cells. *Immunity*, 6(3):235–244, March 1997. ISSN 1074-7613. doi: 10.1016/S1074-7613(00)80326-X. URL http://www.sciencedirect.com/science/article/pii/S107476130080326X.

[692] Stephen J Dollery, Catherine C Wright, David C Johnson, and Anthony V Nicola. Low-pH-dependent changes in the conformation and oligomeric state of the prefusion form of herpes simplex virus glycoprotein b are separable from fusion activity. *Journal of virology*, 85(19):9964–9973, October 2011. ISSN 1098-5514. doi: 10.1128/JVI.05291-11. PMID: 21813610 PMCID: PMC3196434.

[693] Stephen J Dollery, Mark G Delboy, and Anthony V Nicola. Low pH-induced conformational change in herpes simplex virus glycoprotein b. *Journal of virology*, 84(8):3759–3766, April 2010. ISSN 1098-5514. doi: 10.1128/JVI.02573-09. PMID: 20147407 PMCID: PMC2849479.

[694] Carlos R Siekavizza-Robles, Stephen J Dollery, and Anthony V Nicola. Reversible conformational change in herpes simplex virus glycoprotein b with fusion-from-without activity is triggered by mildly acidic pH. *Virology journal*, 7:352, 2010. ISSN 1743-422X. doi: 10.1186/1743-422X-7-352. PMID: 21122119 PMCID: PMC3003269.

[695] Jun Arii, Jing Wang, Tomomi Morimoto, Tadahiro Suenaga, Hiroomi Akashi, Hisashi Arase, and Yasushi Kawaguchi. A Single-Amino-Acid substitution in herpes simplex virus 1 envelope glycoprotein b at a site required for binding to the paired Immunoglobulin-Like type 2 receptor alpha (PILRalpha) abrogates PILRalpha-Dependent viral entry and reduces pathogenesis. *J. Virol.*, 84(20):10773–10783, October 2010. doi: 10.1128/JVI.01166-10.

[696] Takeshi Satoh, Jun Arii, Tadahiro Suenaga, Jing Wang, Amane Kogure, Junji Uehori, Noriko Arase, Ikuo Shiratori, Shinya Tanaka, Yasushi Kawaguchi, Patricia G Spear, Lewis L Lanier, and Hisashi Arase. PILRalpha is a herpes simplex virus-1 entry coreceptor that associates with glycoprotein b. *Cell*, 132(6):935–944, March 2008. ISSN 1097-4172. doi: 10.1016/j.cell.2008.01.043. PMID: 18358807 PMCID: PMC2394663.

[697] Takahiko Imai, Jun Arii, Atsuko Minowa, Aya Kakimoto, Naoto Koyanagi, Akihisa Kato, and Yasushi Kawaguchi. Role of the herpes simplex virus 1 us3 kinase phosphorylation site and endocytosis motifs in the intracellular transport and neurovirulence of envelope glycoprotein b. *Journal of virology*, 85(10):5003–5015, May 2011. ISSN 1098-5514. doi: 10.1128/JVI.02314-10. PMID: 21389132 PMCID: PMC3126194.

[698] Takahiko Imai, Ken Sagou, Jun Arii, and Yasushi Kawaguchi. Effects of phosphorylation of herpes simplex virus 1 envelope glycoprotein b by us3 kinase in vivo and in vitro. *Journal of virology*, 84(1):153–162, January 2010. ISSN 1098-5514. doi: 10.1128/JVI.01447-09. PMID: 19846518 PMCID: PMC2798420.

[699] David Navarro, Pedro Paz, and Lenore Pereira. Domains of herpes simplex virus i glycoprotein b that function in virus penetration, cell-to-cell spread, and cell fusion. *Virology*, 186(1):99–112, January 1992. ISSN 0042-6822. doi: 10.1016/0042-6822(92)90064-V. URL http://www.sciencedirect.com/science/article/pii/004268229290064V.

[700] A Diakidi-Kosta, G Michailidou, G Kontogounis, A Sivropoulou, and M Arsenakis. A single amino acid substitution in the cytoplasmic tail of the glycoprotein b of herpes simplex virus 1 affects both syncytium formation and binding to intracellular heparan sulfate. *Virus research*, 93(1):99–108, May 2003. ISSN 0168-1702. PMID: 12727347.

[701] Igor Beitia Ortiz de Zarate, Lilia Cantero-Aguilar, Magalie Longo, Clarisse Berlioz-Torrent, and Flore Rozenberg. Contribution of endocytic motifs in the cytoplasmic tail of herpes simplex virus type 1 glycoprotein b to virus replication and cell-cell fusion. *Journal of virology*, 81(24):13889–13903, December 2007. ISSN 1098-5514. doi: 10.1128/JVI.01231-07. PMID: 17913800 PMCID: PMC2168835.

[702] Heather M. Coleman, Viv Connor, Zara S. C. Cheng, Finn Grey, Chris M. Preston, and Stacey Efstathiou. Histone modifications associated with herpes simplex virus type 1 genomes during quiescence and following ICP0-mediated de-repression. *Journal of General Virology*, 89(1):68–77, January 2008. ISSN 0022-1317, 1465-2099. doi: 10.1099/vir.0.83272-0. URL http://vir.sgmjournals.org/content/89/1/68.

[703] Anna R. Cliffe and David M. Knipe. Herpes simplex virus ICP0 promotes both histone removal and acetylation on

viral DNA during lytic infection. *Journal of Virology*, 82(24):12030–12038, December 2008. ISSN 0022-538X. doi: 10.1128/JVI.01575-08. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2593313/.

[704] Michael W Ferenczy and Neal A DeLuca. Epigenetic modulation of gene expression from quiescent herpes simplex virus genomes. *Journal of Virology*, 83(17):8514–8524, September 2009. ISSN 1098-5514. doi: 10.1128/JVI.00785-09.

[705] Maria Kalamvoki and Bernard Roizman. ICP0 enables and monitors the function of d cyclins in herpes simplex virus 1 infected cells. *Proceedings of the National Academy of Sciences of the United States of America*, 106 (34):14576–14580, August 2009. ISSN 1091-6490. doi: 10.1073/pnas.0906905106. PMID: 19706544 PMCID: PMC2732861.

[706] Maria Kalamvoki and Bernard Roizman. Interwoven roles of cyclin d3 and cdk4 recruited by ICP0 and ICP4 in the expression of herpes simplex virus genes. *Journal of virology*, 84(19):9709–9717, October 2010. ISSN 1098-5514. doi: 10.1128/JVI.01050-10. PMID: 20660182 PMCID: PMC2937768.

[707] Caroline E. Lilley, Mira S. Chaurushiya, Chris Boutell, Roger D. Everett, and Matthew D. Weitzman. The intrinsic antiviral defense to incoming HSV-1 genomes includes specific DNA repair proteins and is counteracted by the viral protein ICP0. *PLoS Pathog*, 7(6):e1002084, June 2011. doi: 10.1371/journal.ppat.1002084. URL http://dx.doi.org/10.1371/journal.ppat.1002084.

[708] Caroline E Lilley, Mira S Chaurushiya, Chris Boutell, Sebastien Landry, Junghae Suh, Stephanie Panier, Roger D Everett, Grant S Stewart, Daniel Durocher, and Matthew D Weitzman. A viral e3 ligase targets RNF8 and RNF168 to control histone ubiquitination and DNA damage responses. *The EMBO journal*, 29(5):943–955, March 2010. ISSN 1460-2075. doi: 10.1038/emboj.2009.400. PMID: 20075863 PMCID: PMC2837166.

[709] Mirna Perusina Lanfranca, Heba H. Mostafa, and David J. Davido. Two overlapping regions within the n-terminal half of the herpes simplex virus 1 e3 ubiquitin ligase ICP0 facilitate the degradation and dissociation of PML and dissociation of sp100 from ND10. *Journal of Virology*, 87(24):13287–13296, December 2013. ISSN 0022-538X, 1098-5514. doi: 10.1128/JVI.02304-13. URL http://jvi.asm.org/content/87/24/13287. PMID: 24089549.

[710] Shuai Wang, Jing Long, and Chun-fu Zheng. The potential link between PML NBs and ICP0 in regulating lytic and latent infection of HSV-1. *Protein & cell*, 3(5):372–382, May 2012. ISSN 1674-8018. doi: 10.1007/s13238-012-2021-x. PMID: 22544561.

[711] Allison L. van Lint, Matthew R. Murawski, Rory E. Goodbody, Martina Severa, Katherine A. Fitzgerald, Robert W. Finberg, David M. Knipe, and Evelyn A. Kurt-Jones. Herpes simplex virus immediate-early ICP0 protein inhibits toll-like receptor 2-dependent inflammatory responses and NF-?B signaling. *Journal of Virology*, 84(20):10802–10811, October 2010. ISSN 0022-538X. doi: 10.1128/JVI.00063-10. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2950559/.

[712] William P Halford, Carla Weisend, Jennifer Grace, Mark Soboleski, Daniel J J Carr, John W Balliet, Yumi Imai, Todd P Margolis, and Bryan M Gebhardt. ICP0 antagonizes stat 1-dependent repression of herpes simplex virus: implications for the regulation of viral latency. *Virology journal*, 3:44, 2006. ISSN 1743-422X. doi: 10.1186/1743-422X-3-44. PMID: 16764725 PMCID: PMC1557838.

[713] K L Mossman, H A Saffran, and J R Smiley. Herpes simplex virus ICP0 mutants are hypersensitive to interferon. *Journal of virology*, 74(4):2052–2056, February 2000. ISSN 0022-538X. PMID: 10644380 PMCID: PMC111685.

[714] K L Mossman, P F Macgregor, J J Rozmus, A B Goryachev, A M Edwards, and J R Smiley. Herpes simplex virus triggers and then disarms a host antiviral response. *Journal of virology*, 75(2):750–758, January 2001. ISSN 0022-538X. doi: 10.1128/JVI.75.2.750-758.2001. PMID: 11134288 PMCID: PMC113971.

[715] Rongtuan Lin, Ryan S. Noyce, Susan E. Collins, Roger D. Everett, and Karen L. Mossman. The herpes simplex virus ICP0 RING finger domain inhibits IRF3- and IRF7-Mediated activation of Interferon-Stimulated genes. *J. Virol.*, 78(4):1675–1684, February 2004. doi: 10.1128/JVI.78.4.1675-1684.2004.

[716] Kasey M. Eidson, William E. Hobbs, Brian J. Manning, Paul Carlson, and Neal A. DeLuca. Expression of herpes

simplex virus ICP0 inhibits the induction of Interferon-Stimulated genes by viral infection. *J. Virol.*, 76(5):2180–2191, March 2002. doi: 10.1128/jvi.76.5.2180-2191.2002.

[717] Kathryne E Taylor, Marianne V Chew, Ali A Ashkar, and Karen L Mossman. Novel roles of cytoplasmic ICP0: proteasome-independent functions of the RING finger are required to block ISG production but not to promote viral replication. *Journal of virology*, May 2014. ISSN 1098-5514. doi: 10.1128/JVI.00944-14. PMID: 24807717.

[718] Patrick Paladino, Susan E Collins, and Karen L Mossman. Cellular localization of the herpes simplex virus ICP0 protein dictates its ability to block IRF3-mediated innate immune responses. *PloS one*, 5(4):e10428, 2010. ISSN 1932-6203. doi: 10.1371/journal.pone.0010428. PMID: 20454685 PMCID: PMC2861674.

[719] Megan H Orzalli, Neal A DeLuca, and David M Knipe. Nuclear IFI16 induction of IRF-3 signaling during herpesviral infection and degradation of IFI16 by the viral ICP0 protein. *Proceedings of the National Academy of Sciences of the United States of America*, 109(44):E3008–3017, October 2012. ISSN 1091-6490. doi: 10.1073/pnas.1211302109. PMID: 23027953 PMCID: PMC3497734.

[720] Christiane S Heilingloh, Petra Mhl-Zrbes, Alexander Steinkasserer, and Mirko Kummer. ICP0 induces CD83-degradation in mature dendritic cells independently of its e3 ubiquitin ligase function. *The Journal of general virology*, March 2014. ISSN 1465-2099. doi: 10.1099/vir.0.062810-0. PMID: 24643878.

[721] Mirko Kummer, Nadine M Turza, Petra Muhl-Zurbes, Matthias Lechmann, Chris Boutell, Robert S Coffin, Roger D Everett, Alexander Steinkasserer, and Alexander T Prechtel. Herpes simplex virus type 1 induces CD83 degradation in mature dendritic cells with immediate-early kinetics via the cellular proteasome. *Journal of virology*, 81(12): 6326–6338, June 2007. ISSN 0022-538X. doi: 10.1128/JVI.02327-06. PMID: 17428858 PMCID: PMC1900083.

[722] D Kobelt, M Lechmann, and A Steinkasserer. The interaction between dendritic cells and herpes simplex virus-1. *Current topics in microbiology and immunology*, 276:145–161, 2003. ISSN 0070-217X. PMID: 12797447.

[723] Mingyu Liu, Edward E Schmidt, and William P Halford. ICP0 dismantles microtubule networks in herpes simplex virus-infected cells. *PloS one*, 5(6):e10975, 2010. ISSN 1932-6203. doi: 10.1371/journal.pone.0010975. PMID: 20544015 PMCID: PMC2882321.

[724] Susan E Chisholm, Keith Howard, Mar Vals Gmez, and Hugh T Reyburn. Expression of ICP0 is sufficient to trigger natural killer cell recognition of herpes simplex virus-infected cells by natural cytotoxicity receptors. *The Journal of infectious diseases*, 195(8):1160–1168, April 2007. ISSN 0022-1899. doi: 10.1086/512862.

[725] Mark G. Delboy and Anthony V. Nicola. A pre-immediate-early role for tegument ICP0 in the proteasome-dependent entry of herpes simplex virus. *Journal of Virology*, 85(12):5910–5918, June 2011. ISSN 0022-538X. doi: 10.1128/JVI.00267-11. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3126318/.

[726] B J Biegalke and A P Geballe. Sequence requirements for activation of the HIV-1 LTR by human cytomegalovirus. *Virology*, 183(1):381–385, July 1991. ISSN 0042-6822. PMID: 1647074.

[727] P A Barry, E Pratt-Lowe, B M Peterlin, and P A Luciw. Cytomegalovirus activates transcription directed by the long terminal repeat of human immunodeficiency virus type 1. *Journal of virology*, 64(6):2932–2940, June 1990. ISSN 0022-538X. PMID: 2159554 PMCID: PMC249477.

[728] M Gmez-Gonzalo, M Carretero, J Rullas, E Lara-Pezzi, J Aramburu, B Berkhout, J Alcam, and M Lpez-Cabrera. The hepatitis b virus x protein induces HIV-1 replication and transcription in synergy with t-cell activation signals: functional roles of NF-kappaB/NF-AT and SP1-binding sites in the HIV-1 long terminal repeat promoter. *The Journal of biological chemistry*, 276(38):35435–35443, September 2001. ISSN 0021-9258. doi: 10.1074/jbc.M103020200. PMID: 11457829.

[729] S F Parker, L K Felzien, N D Perkins, M J Imperiale, and G J Nabel. Distinct domains of adenovirus E1A interact with specific cellular factors to differentially modulate human immunodeficiency virus transcription. *Journal of virology*, 71(3):2004–2012, March 1997. ISSN 0022-538X. PMID: 9032332 PMCID: PMC191285.

[730] Satarupa Sengupta, Eleanor Powell, Ling Kong, and Jason T Blackard. Effects of HCV on basal and tat-induced

HIV LTR activation. *PloS one*, 8(6):e64956, 2013. ISSN 1932-6203. doi: 10.1371/journal.pone.0064956. PMID: 23762271 PMCID: PMC3677892.

[731] Lei Kang, Zhen Luo, Youxing Li, Wenjing Zhang, Wei Sun, Wei Li, Yanni Chen, Fang Liu, Xueshan Xia, Ying Zhu, and Jianguo Wu. Association of vpu with hepatitis c virus NS3/4A stimulates transcription of type 1 human immunodeficiency virus. *Virus research*, 163(1):74–81, January 2012. ISSN 1872-7492. doi: 10.1016/j.virusres.2011.08.011. PMID: 21889553.

[732] Xiaoli Yu, Zhanqiang Sun, Chenjun Zhou, Zilu Wen, Jun Chen, Qingwen Sun, Honghai Wang, and Shulin Zhang. [expression, purification, and characterization mycobacterium tuberculosis rv1168c]. *Wei sheng wu xue bao = Acta microbiologica Sinica*, 50(7):931–936, July 2010. ISSN 0001-6209. PMID: 20815241.

[733] R. J. Pomerantz, M. B. Feinberg, D. Trono, and D. Baltimore. Lipopolysaccharide is a potent monocyte/macrophage-specific stimulator of human immunodeficiency virus type 1 expression. *The Journal of Experimental Medicine*, 172(1):253–261, July 1990. ISSN 0022-1007, 1540-9538. doi: 10.1084/jem.172.1.253. URL http://jem.rupress.org/content/172/1/253. PMID: 2193097.

[734] L Xiao, S M Owen, D L Rudolph, R B Lal, and A A Lal. Plasmodium falciparum antigen-induced human immunodeficiency virus type 1 replication is mediated through induction of tumor necrosis factor-alpha. *The Journal of infectious diseases*, 177(2):437–445, February 1998. ISSN 0022-1899. PMID: 9466533.

[735] R. Bernier, S. J. Turco, M. Olivier, and M. Tremblay. Activation of human immunodeficiency virus type 1 in monocytoid cells by the protozoan parasite leishmania donovani. *Journal of Virology*, 69(11):7282–7285, November 1995. ISSN 0022-538X, 1098-5514. URL http://jvi.asm.org/content/69/11/7282. PMID: 7474154.

[736] Juan A Recio, Jorge Mart?nez de la Mata, Jos Mart?n-Nieto, and Ana Aranda. Retinoic acid stimulates HIV-1 transcription in human neuroblastoma SH-SY5Y cells. *FEBS Letters*, 469(1):118–122, March 2000. ISSN 0014-5793. doi: 10.1016/S0014-5793(00)01249-7. URL http://www.sciencedirect.com/science/article/pii/S0014579300012497.

[737] Julin Nevado, Stephan P Tenbaum, Ana Isabel Castillo, Aurora Snchez-Pacheco, and Ana Aranda. Activation of the human immunodeficiency virus type i long terminal repeat by 1 alpha,25-dihydroxyvitamin d3. *Journal of molecular endocrinology*, 38(6):587–601, June 2007. ISSN 1479-6813. doi: 10.1677/JME-06-0065. PMID: 17556530.

[738] Lei Guo, Wen-juan Wu, Long-ding Liu, Li-chun Wang, Ying Zhang, Lian-qiu Wu, Ying Guan, and Qi-han Li. Herpes simplex virus 1 ICP22 inhibits the transcription of viral gene promoters by binding to and blocking the recruitment of p-TEFb. *PLoS ONE*, 7(9):e45749, September 2012. doi: 10.1371/journal.pone.0045749. URL http://dx.doi.org/10.1371/journal.pone.0045749.

[739] Y-T. Wu, H-L. Tan, Q. Huang, X-J. Sun, X. Zhu, and H-M. Shen. zvad-induced necroptosis in l929 cells depends on autocrine production of tnf? mediated by the pkc-mapks-ap-1 pathway. *Cell Death Differ*, 18(1):26–37, Jan 2011. doi: 10.1038/cdd.2010.72. URL http://dx.doi.org/10.1038/cdd.2010.72.

[740] Mark Ou and Rozanne M. Sandri-Goldin. Inhibition of cdk9 during herpes simplex virus 1 infection impedes viral transcription. *PLoS ONE*, 8(10), October 2013. ISSN 1932-6203. doi: 10.1371/journal.pone.0079007. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3799718/.

[741] Lizette Olga Durand and Bernard Roizman. Role of cdk9 in the optimization of expression of the genes regulated by ICP22 of herpes simplex virus 1. *Journal of Virology*, 82(21):10591–10599, November 2008. ISSN 0022-538X. doi: 10.1128/JVI.01242-08. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2573194/.

[742] S. Millhouse, J. J. Kenny, P. G. Quinn, V. Lee, and B. Wigdahl. ATF/CREB elements in the herpes simplex virus type 1 latency-associated transcript promoter interact with members of the ATF/CREB and AP-1 transcription factor families. *Journal of Biomedical Science*, 5(6):451–464, December 1998. ISSN 1021-7770.

[743] K. L. Jang, B. Pulverer, J. R. Woodgett, and D. S. Latchman. Activation of the cellular transcription factor AP-1 in herpes simplex virus infected cells is dependent on the viral immediate-early protein ICPO. *Nucleic Acids Research*, 19(18):4879–4883, September 1991. ISSN 0305-1048.

[744] E. S. Scott, S. Malcomber, and P. O'Hare. Nuclear translocation and activation of the transcription factor NFAT is blocked by herpes simplex virus infection. *Journal of Virology*, 75(20):9955–9965, October 2001. ISSN 0022-538X. doi: 10.1128/JVI.75.20.9955-9965.2001.

[745] F B Knotts, M L Cook, and J G Stevens. Latent herpes simplex virus in the central nervous system of rabbits and mice. *The Journal of experimental medicine*, 138(3):740–744, September 1973. ISSN 0022-1007. PMID: 4353820 PMCID: PMC2139419.

[746] G Plummer, C R Goodheart, M Miyagi, G R Skinner, M E Thouless, and P Wildy. Herpes simplex viruses: discrimination of types and correlation between different characteristics. *Virology*, 60(1):206–216, July 1974. ISSN 0042-6822. PMID: 4366799.

[747] W P Halford, B M Gebhardt, and D J Carr. Mechanisms of herpes simplex virus type 1 reactivation. *Journal of Virology*, 70(8):5051–5060, August 1996. ISSN 0022-538X. URL `http://www.ncbi.nlm.nih.gov/pmc/articles/PMC190459/`. PMID: 8764012 PMCID: PMC190459.

[748] Te Du, Guoying Zhou, and Bernard Roizman. HSV-1 gene expression from reactivated ganglia is disordered and concurrent with suppression of latency-associated transcript and miRNAs. *Proceedings of the National Academy of Sciences of the United States of America*, 108(46):18820–18824, November 2011. ISSN 1091-6490. doi: 10.1073/pnas.1117203108. PMID: 22065742 PMCID: PMC3219146.

[749] R Tal-Singer, T M Lasner, W Podrzucki, A Skokotas, J J Leary, S L Berger, and N W Fraser. Gene expression during reactivation of herpes simplex virus type 1 from latency in the peripheral nervous system is different from that during lytic infection of tissue cultures. *Journal of Virology*, 71(7):5268–5276, July 1997. ISSN 0022-538X. URL `http://www.ncbi.nlm.nih.gov/pmc/articles/PMC191763/`. PMID: 9188595 PMCID: PMC191763.

[750] C Shimeld, T Hill, B Blyth, and D Easty. An improved model of recurrent herpetic eye disease in mice. *Current eye research*, 8(11):1193–1205, November 1989. ISSN 0271-3683. PMID: 2558849.

[751] N M Sawtell and R L Thompson. Rapid in vivo reactivation of herpes simplex virus in latently infected murine ganglionic neurons after transient hyperthermia. *Journal of virology*, 66(4):2150–2156, April 1992. ISSN 0022-538X. PMID: 1312625 PMCID: PMC289007.

[752] N M Sawtell. Quantitative analysis of herpes simplex virus reactivation in vivo demonstrates that reactivation in the nervous system is not inhibited at early times postinoculation. *Journal of virology*, 77(7):4127–4138, April 2003. ISSN 0022-538X. PMID: 12634371 PMCID: PMC150616.

[753] M E Marquart, X Zheng, R K Tran, H W Thompson, D C Bloom, and J M Hill. A cAMP response element within the latency-associated transcript promoter of HSV-1 facilitates induced ocular reactivation in a mouse hyperthermia model. *Virology*, 284(1):62–69, May 2001. ISSN 0042-6822. doi: 10.1006/viro.2001.0911. PMID: 11352668.

[754] J M Hill, J B Dudley, Y Shimomura, and H E Kaufman. Quantitation and kinetics of induced HSV-1 ocular shedding. *Current eye research*, 5(3):241–246, March 1986. ISSN 0271-3683. PMID: 3009094.

[755] Y J Gordon, E Romanowski, and T Araullo-Cruz. A fast, simple reactivation method for the study of HSV-1 latency in the rabbit ocular model. *Investigative ophthalmology & visual science*, 31(5):921–924, May 1990. ISSN 0146-0404. PMID: 2159452.

[756] W A Blyth, D A Harbour, and T J Hill. Effect of immunosuppression on recurrent herpes simplex in mice. *Infection and immunity*, 29(3):902–907, September 1980. ISSN 0019-9567. PMID: 6253401 PMCID: PMC551216.

[757] S D Cook, M J Paveloff, J J Doucet, A J Cottingham, F Sedarati, and J M Hill. Ocular herpes simplex virus reactivation in mice latently infected with latency-associated transcript mutants. *Investigative ophthalmology & visual science*, 32(5):1558–1561, April 1991. ISSN 0146-0404. PMID: 1849874.

[758] Shiro Higaki, Bryan M Gebhardt, Walter J Lukiw, Hilary W Thompson, and James M Hill. Effect of immunosuppression on gene expression in the HSV-1 latently infected mouse trigeminal ganglion. *Investigative ophthalmology & visual science*, 43(6):1862–1869, June 2002. ISSN 0146-0404. PMID: 12036991.

[759] I Zlotnik, C E Smith, D P Grant, and S Peacock. The effect of immunosuppression on viral encephalitis, with special reference to cyclophosphamide. *British journal of experimental pathology*, 51(4):434–439, August 1970. ISSN 0007-1021. PMID: 4991966 PMCID: PMC2072316.

[760] David A. Padgett, John F. Sheridan, Julianne Dorne, Gary G. Berntson, Jessica Candelora, and Ronald Glaser. Social stress and the reactivation of latent herpes simplex virus type 1. *Proceedings of the National Academy of Sciences of the United States of America*, 95(12):7231–7235, June 1998. ISSN 0027-8424. URL `http://www.ncbi.nlm.nih.gov/pmc/articles/PMC22787/`. PMID: 9618568 PMCID: PMC22787.

[761] Ju Youn Kim, Angelo Mandarino, Moses V Chao, Ian Mohr, and Angus C Wilson. Transient reversal of episome silencing precedes VP16-dependent transcription during reactivation of latent HSV-1 in neurons. *PLoS pathogens*, 8(2):e1002540, February 2012. ISSN 1553-7374. doi: 10.1371/journal.ppat.1002540. PMID: 22383875 PMCID: PMC3285597.

[762] Vladimir Camarena, Mariko Kobayashi, Ju Youn Kim, Pamela Roehm, Rosalia Perez, James Gardner, Angus C Wilson, Ian Mohr, and Moses V Chao. Nature and duration of growth factor signaling through receptor tyrosine kinases regulates HSV-1 latency in neurons. *Cell host & microbe*, 8(4):320–330, October 2010. ISSN 1934-6069. doi: 10.1016/j.chom.2010.09.007. PMID: 20951966 PMCID: PMC2988476.

[763] C. L. Wilcox, R. L. Smith, C. R. Freed, and E. M. Johnson. Nerve growth factor-dependence of herpes simplex virus latency in peripheral sympathetic and sensory neurons in vitro. *The Journal of Neuroscience*, 10(4):1268–1275, April 1990. ISSN 0270-6474, 1529-2401. URL `http://www.jneurosci.org/content/10/4/1268`. PMID: 2158529.

[764] S Syrjnen, H Mikola, M Nyknen, and V Hukkanen. In vitro establishment of lytic and nonproductive infection by herpes simplex virus type 1 in three-dimensional keratinocyte culture. *Journal of virology*, 70(9):6524–6528, September 1996. ISSN 0022-538X. PMID: 8709294 PMCID: PMC190692.

[765] Alexandra Kataropoulou, Chiara Bovolenta, Amalia Belfiore, Sonia Trabatti, Anna Garbelli, Simona Porcellini, Rossella Lupo, and Giovanni Maga. Mutational analysis of the HIV-1 auxiliary protein vif identifies independent domains important for the physical and functional interaction with HIV-1 reverse transcriptase. *Nucleic Acids Research*, 37(11):3660–3669, June 2009. ISSN 1362-4962. doi: 10.1093/nar/gkp226.

[766] Reynel Cancio, Silvio Spadari, and Giovanni Maga. Vif is an auxiliary factor of the HIV-1 reverse transcriptase and facilitates abasic site bypass. *The Biochemical Journal*, 383(Pt. 3):475–482, November 2004. ISSN 1470-8728. doi: 10.1042/BJ20040914.

[767] N. K. Heinzinger, M. I. Bukinsky, S. A. Haggerty, A. M. Ragland, V. Kewalramani, M. A. Lee, H. E. Gendelman, L. Ratner, M. Stevenson, and M. Emerman. The vpr protein of human immunodeficiency virus type 1 influences nuclear localization of viral nucleic acids in nondividing host cells. *Proceedings of the National Academy of Sciences of the United States of America*, 91(15):7311–7315, July 1994. ISSN 0027-8424.

[768] Biswanath Majumder, Michelle L. Janket, Elizabeth A. Schafer, Keri Schaubert, Xiao-Li Huang, June Kan-Mitchell, Charles R. Rinaldo, and Velpandi Ayyavoo. Human immunodeficiency virus type 1 vpr impairs dendritic cell maturation and t-cell activation: implications for viral immune escape. *Journal of Virology*, 79(13):7990–8003, July 2005. ISSN 0022-538X. doi: 10.1128/JVI.79.13.7990-8003.2005.

[769] Karuppiah Muthumani, Andrew Y. Choo, Daniel S. Hwang, Arumugam Premkumar, Nathanael S. Dayes, Crafford Harris, Douglas R. Green, Scott A. Wadsworth, John J. Siekierka, and David B. Weiner. HIV-1 nef-induced FasL induction and bystander killing requires p38 MAPK activation. *Blood*, 106(6):2059–2068, September 2005. ISSN 0006-4971. doi: 10.1182/blood-2005-03-0932. URL `http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1895138/`.

[770] Narasimhan J. Venkatachari, Biswanath Majumder, and Velpandi Ayyavoo. Human immunodeficiency virus (HIV) type 1 vpr induces differential regulation of t cell costimulatory molecules: Direct effect of vpr on t cell activation and immune function. *Virology*, 358(2):347–356, February 2007. ISSN 0042-6822. doi: 10.1016/j.virol.2006.08.030. URL `http://www.sciencedirect.com/science/article/pii/S004268220600599X`.

[771] Jonathan Richard, Sardar Sindhu, Tram N. Q. Pham, Jean-Philippe Belzile, and Eric A. Cohen. HIV-1 vpr upregulates expression of ligands for the activating NKG2d receptor and promotes NK cell-mediated killing. *Blood*, 115(7):1354–1363, February 2010. ISSN 1528-0020. doi: 10.1182/blood-2009-08-237370.

[772] Jeffrey Ward, Zachary Davis, Jason DeHart, Erik Zimmerman, Alberto Bosque, Enrico Brunetta, Domenico Mavilio, Vicente Planelles, and Edward Barker. HIV-1 vpr triggers natural killer cellmediated lysis of infected cells through activation of the ATR-mediated DNA damage response. *PLoS Pathog*, 5(10):e1000613, October 2009. doi: 10.1371/journal.ppat.1000613. URL http://dx.doi.org/10.1371/journal.ppat.1000613.

[773] J. V. Garcia and A. D. Miller. Serine phosphorylation-independent downregulation of cell-surface CD4 by nef. *Nature*, 350(6318):508–511, April 1991. ISSN 0028-0836. doi: 10.1038/350508a0.

[774] Prabha Chandrasekaran, Victoria Moore, Monica Buckley, Joshua Spurrier, John H. Kehrl, and Sundararajan Venkatesan. HIV-1 nef down-modulates c-c and c-x-c chemokine receptors via ubiquitin and ubiquitin-independent mechanism. *PloS One*, 9(1):e86998, 2014. ISSN 1932-6203. doi: 10.1371/journal.pone.0086998.

[775] Luciana J. Costa, Nan Chen, Adriana Lopes, Renato S. Aguiar, Amilcar Tanuri, Ana Plemenitas, and B. Matija Peterlin. Interactions between nef and AIP1 proliferate multivesicular bodies and facilitate egress of HIV-1. *Retrovirology*, 3:33, 2006. ISSN 1742-4690. doi: 10.1186/1742-4690-3-33.

[776] C. Aiken and D. Trono. Nef stimulates human immunodeficiency virus type 1 proviral DNA synthesis. *Journal of Virology*, 69(8):5048–5056, August 1995. ISSN 0022-538X.

[777] O. Schwartz, V. Marchal, O. Danos, and J. M. Heard. Human immunodeficiency virus type 1 nef increases the efficiency of reverse transcription in the infected cell. *Journal of Virology*, 69(7):4053–4059, July 1995. ISSN 0022-538X.

[778] Luiza M. Mendona, Sandro C. Poeys, Celina M. Abreu, Amilcar Tanuri, and Luciana J. Costa. HIV-1 nef inhibits protease activity and its absence alters protein content of mature viral particles. *PLoS ONE*, 9(4):e95352, April 2014. doi: 10.1371/journal.pone.0095352. URL http://dx.doi.org/10.1371/journal.pone.0095352.

[779] Eleonora Olivetta, Donatella Pietraforte, Ilaria Schiavoni, Maurizio Minetti, Maurizio Federico, and Massimo Sanchez. HIV-1 nef regulates the release of superoxide anions from human macrophages. *Biochemical Journal*, 390(Pt 2):591–602, September 2005. ISSN 0264-6021. doi: 10.1042/BJ20042139. URL http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1198939/.

[780] S. Swingler, J. Jacqu, B. Brichacek, V. G. Sasseville, K. Williams, A. A. Lackner, E. N. Janoff, R. Wang, D. Fisher, and M. Stevenson. HIV-1 nef mediates lymphocyte chemotaxis and activation by infected macrophages. *Nature Medicine*, 5(9):997–103, September 1999. ISSN 1078-8956. doi: 10.1038/12433.

[781] Madeeha Aqil, Afsar Raza Naqvi, Saurav Mallik, Sanghamitra Bandyopadhyay, Ujjwal Maulik, and Shahid Jameel. The HIV nef protein modulates cellular and exosomal miRNA profiles in human monocytic cells. *Journal of Extracellular Vesicles*, 3(0), March 2014. ISSN 2001-3078. doi: 10.3402/jev.v3.23129. URL http://www.journalofextracellularvesicles.net/index.php/jev/article/view/23129.

[782] Madeeha Aqil, Afsar Raza Naqvi, Aalia Shahr Bano, and Shahid Jameel. The HIV-1 nef protein binds argonaute-2 and functions as a viral suppressor of RNA interference. *PloS One*, 8(9):e74472, 2013. ISSN 1932-6203. doi: 10.1371/journal.pone.0074472.

[783] Qin Yan, Xinting Ma, Chenyou Shen, Xu Cao, Ninghan Feng, Di Qin, Yi Zeng, Jianzhong Zhu, Shou-Jiang Gao, and Chun Lu. Inhibition of kaposi's sarcoma-associated herpesvirus lytic replication by HIV-1 nef and cellular microRNA hsa-miR-1258. *Journal of Virology*, 88(9):4987–5000, May 2014. ISSN 1098-5514. doi: 10.1128/JVI.00025-14.

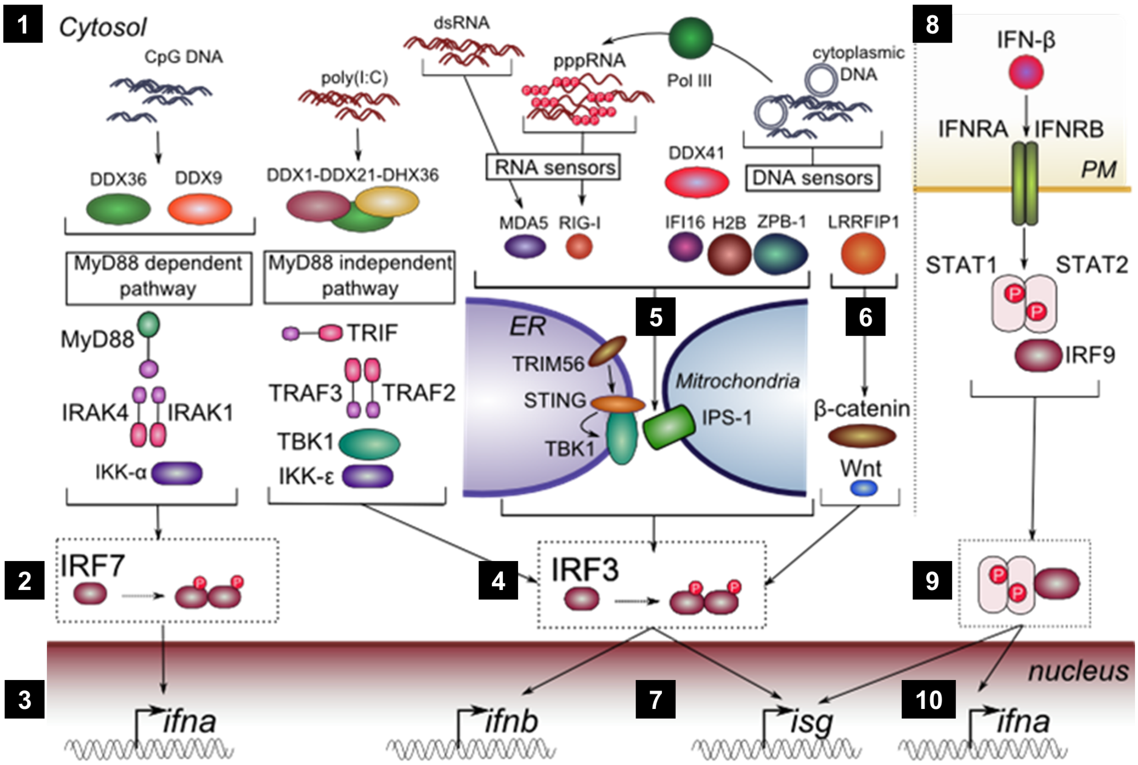

Figure 1: Cytoplasmic PRRs and the type I IFN response.
Numerous nucleic acid sensors detect RNA and/or DNA in the cytoplasm. Synthetic CpG DNA is detected by DDX36 and DDX9, whereas synthetic RNA poly(I:C) is detected by a complex of DDX1, DDX21 and DDX36. [2] DDX36 and DDX9 activate the MyD88 signalling pathway, involving MyD88, IRAK4, IRAK1 and IKK-α, leading to phosphorylation, dimerisation and nuclear translocation of IRF7. [3] IRF7 promotes transcription of the IFN-α genes. [4] DDX1-DDX21-DDX36 activates a MyD88-independent signalling, involving TRIF, TRAF2, TRAF3 and IKK-ϵ, leading to phosphorylation, dimerisation and nuclear translocation of IRF3. [5] IRF3 can also be activated by the STING signalling pathway, involving TRIM56, TBK1 and IPS-1/MAVS, and resulting in an IRF3 activating complex within the mitochondrial membrane. The RNA sensors RIG-I and Mda5 and the DNA sensors DDX41, IFI16, H2B and ZBP-1 all signal this way. [6] The DNA sensor LRRFIP1 activates IRF3 via STING-independent β-catenin signalling. [7] Following nuclear translocation, IRF3 activates transcription of the IFN-β gene, *ifnb*. IRF3 activation can also lead to transcription of ISG, independently of IFN-β transcription. [8] IFN-β is released from the cell and binds type I IFN receptors on the cell surface, in an autocrine or paracrine fashion. [9-10] Ligation of the IFNR activates STAT1/STAT2 phosphorylation, translocation to the nucleus and STAT1/STAT2 dependent induction of ISG and IFN-α gene transcription.

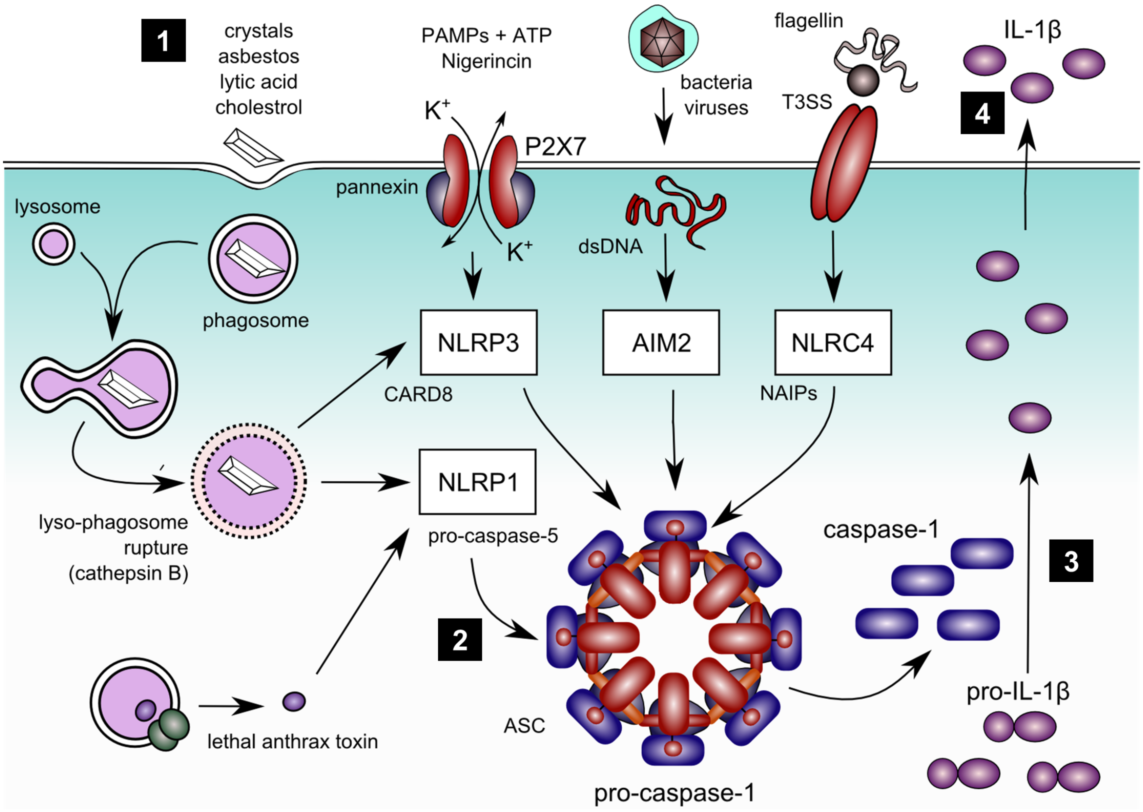

Figure 2: Initiation of pyroptosis.
[1] A variety of danger signals induce formation of a variety of inflammasome complexes. The NLRP3 and NLRP1 inflammasomes are activated by rupture from phago-lysosomes of, for example, crystals and asbestos. NLRP1 is also activated by lethal anthrax toxin and NLRP3 by binding of ATP to the PX2Y receptor. The AIM2 inflammasome is activated by cytosolic dsDNA derived from bacteria and viruses. The NLRC4 inflammasome is activated by detection of flagellin by the T3SS receptor. [2] Inflammasome complexes are multiprotein oligmers consisiting of caspase-1, ASC and either a NLR or AIM2. They can also contain caspase-5, NAIPs or CARD8 in the cases of the the NLRP1, NLRP3 and NLRC4 inflammasomes respectively. [3] Formation of an inflammasome leads to cleavage of pro-caspase-1 into caspase-1. Caspase-1 cleaves pro-IL-1β within the cytoplasm into IL-1β. [4] IL-1β is released from the cell.

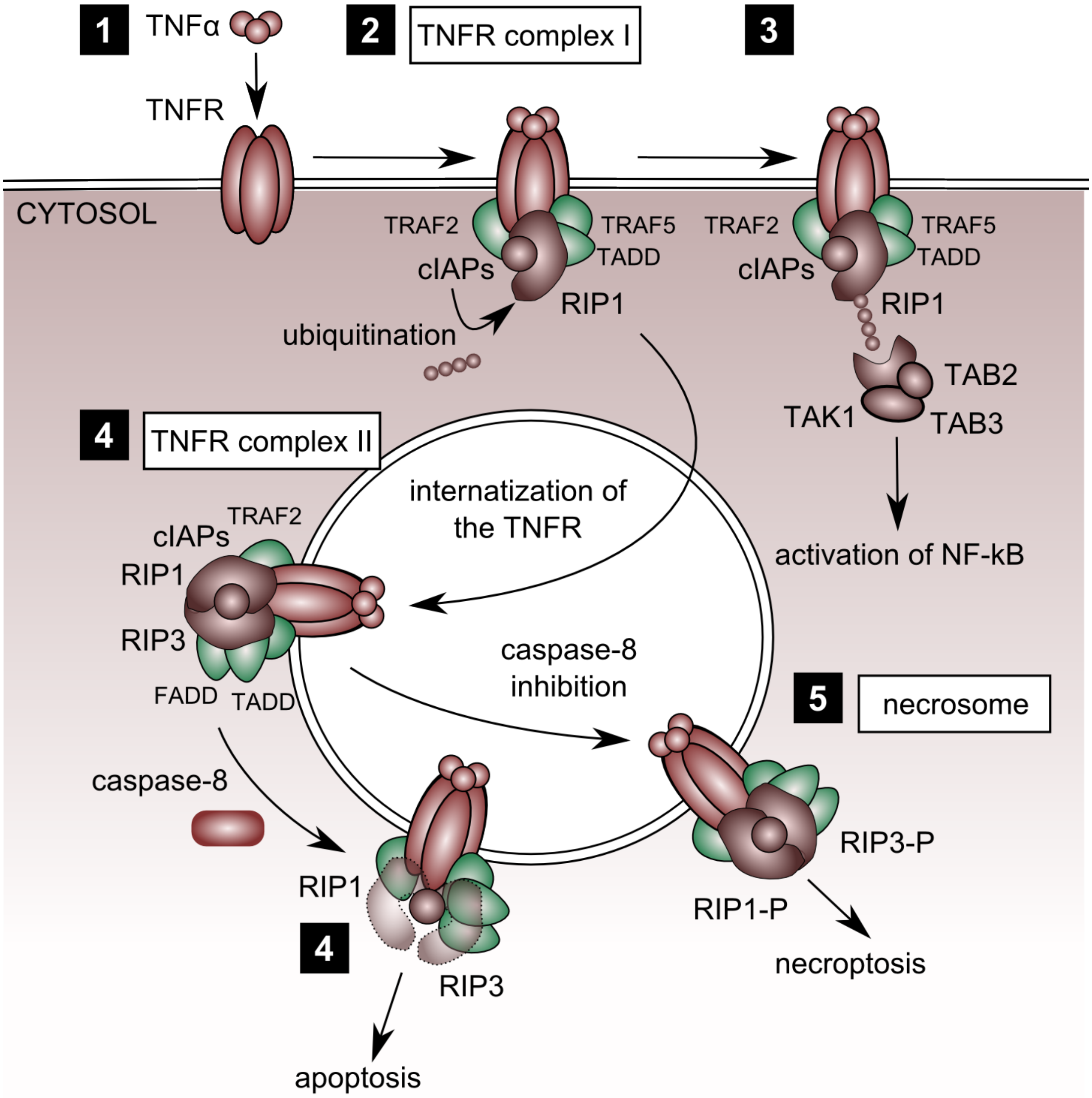

Figure 3: Initiation of necroptosis.
[1] Necroptosis can be initiated by ligation of death receptors, such as TNFR, on the cell surface. [2] Binding of TNF-α to TNFR leads to the formation of the multi-protein TNFR complex I at the cytoplasmic domain of the receptor, containing TRAF2, TRAF5, TADD, cIAPs and RIP1. cIAPs poly-ubiquintinate RIP1. [3] Ubiquinated RIP1 recruits TBK1, TAB2 and TAB3, leading to the initiation of NF-κB signalling. [4] TNFR complex II is formed following internatization of ligated TNFR and the recruitment of RIP3, FADD and caspase-8. [5] Caspase-8 degrades RIP1 and RIP3 and thereby initiates extrinsic apoptosis. [6] If caspase-8 is inhibited RIP1 and RIP3 are phosphorylated, form the necrosome complex and initiate necroptotic signalling.

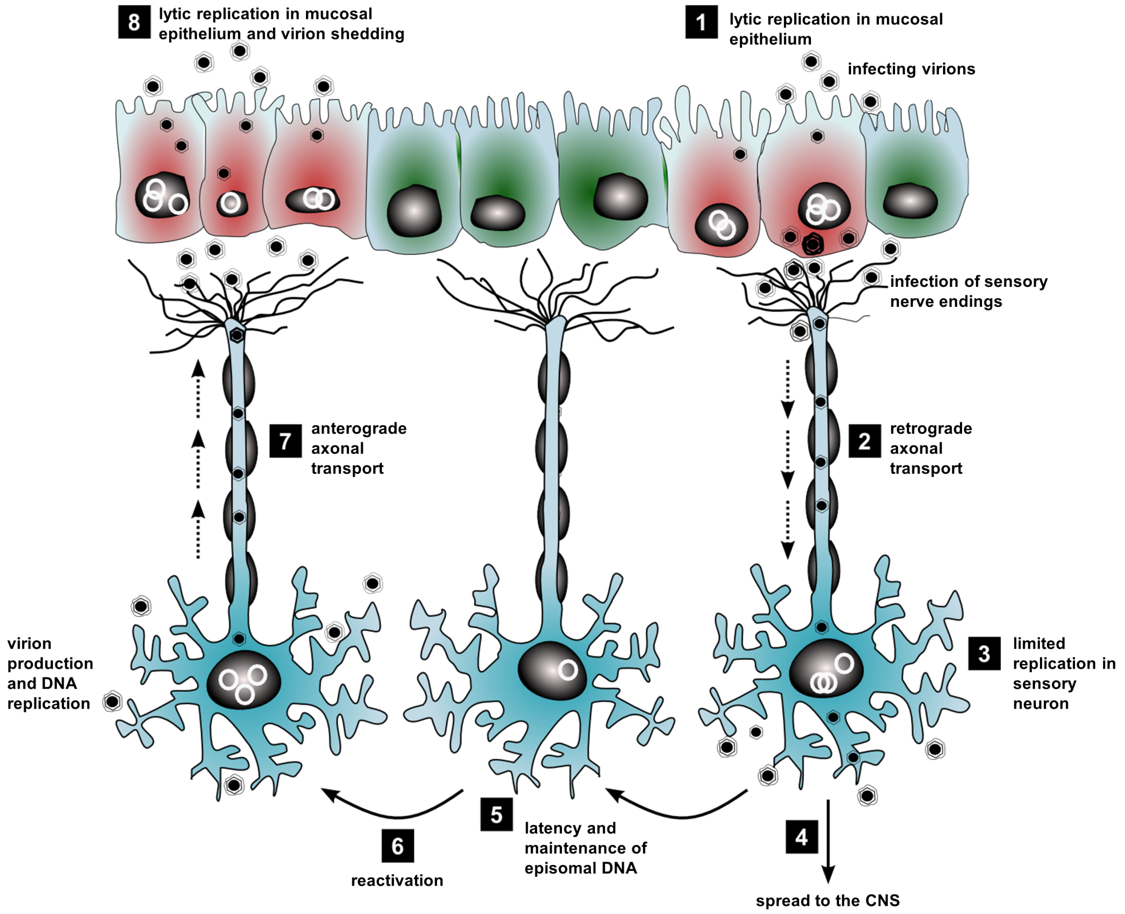

Figure 4: The replication cycle of HSV-1.
HSV-1 transmission occurs at the mucosal epithelium. Following virion entry in the epithelium cells, HSV-1 IE, E and late gene expression and viral DNA replication occurs, followed by release of nascent virions. [2] Infectious virions produced at the site of transmission enter nerve termini of the peripheral nervous system and virial capsids are transported up the axon to the cell body and nucleus(543). [3] HSV-1 lytic replication occurs for a short time in the sensory neurons before latency is established. [4] HSV-1 infection can spread into the CNS, leading to HSV-1 encephalitis. [5] HSV-1 genomes are stably maintained in neurons in the absence of virion production. [6] HSV re-activates in response to environmental and emotional stress(544). [7] Following reactivation HSV-1 virions are transported down the axon(543). [8] HSV-1 reactivation can lead to HSV-1 lesions and virion shedding(159).

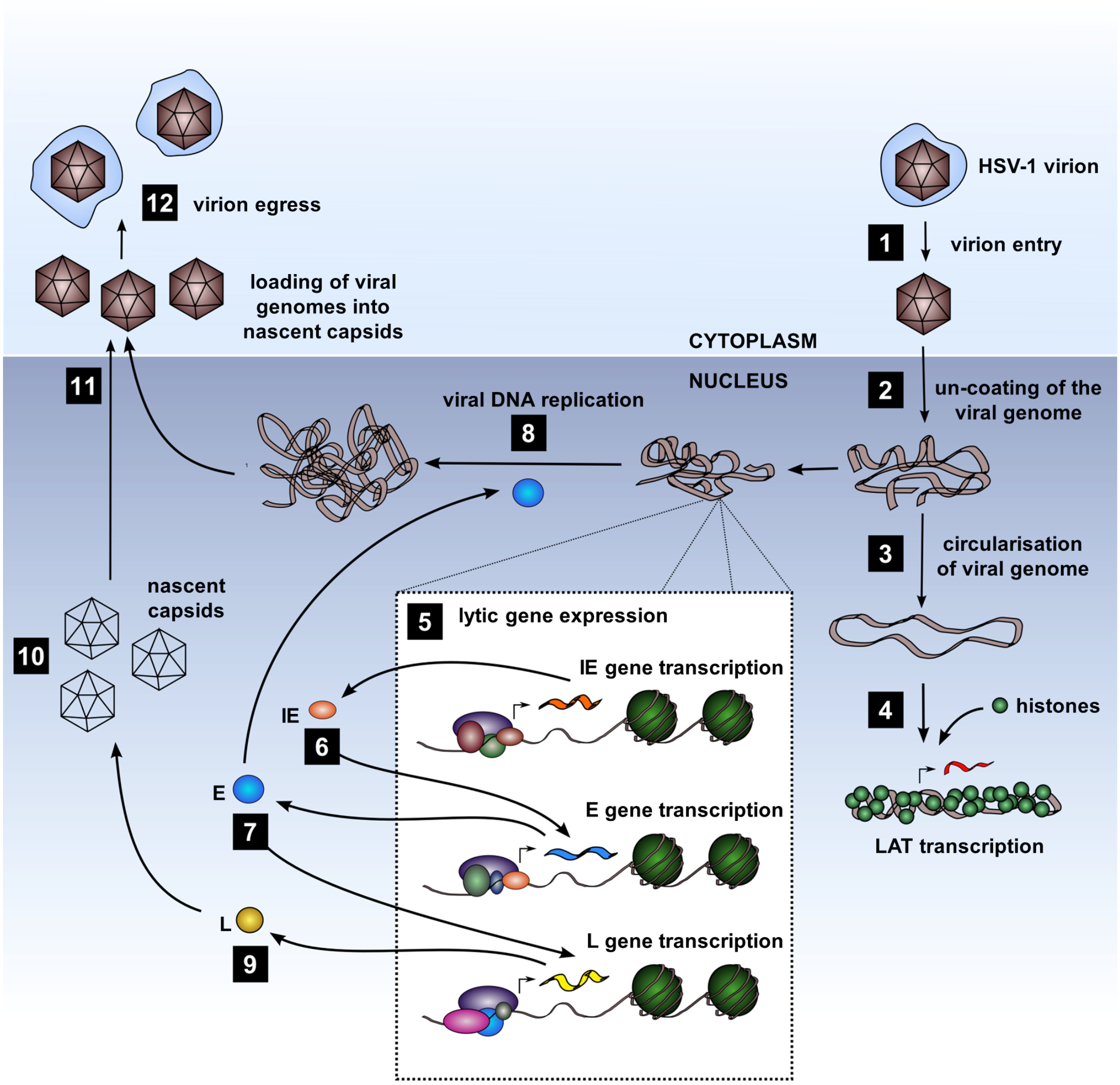

Figure 5: The HSV-1 lytic replication cycle.
[1] HSV-1 virions enter the cell by fusion of cellular membranes and the virion envelope. [2] The viral genome un-coats from the virion capsid and enters the nucleus. [3-4] During latent infection, the viral genome is circularised and maintained in a heterochromatic state such that lytic gene expression is silenced. LAT is the only gene expressed. [5] During lytic infection, IE, E and L genes are expressed sequentially. [6] The virion protein VP16 activates ICP0 transcription. ICP0 promotes expression of the other IE protein and promotes cell survival. IE proteins activate E gene expression. [7] The E gene DNA Pol is responsible for replication of the viral genome. [8-9] Other E proteins activate L gene expression. [10] L proteins include the structural components used to synthesize nascent virion capsids. [11-12] *De novo* viral genomes are packaged into the capsids and other L proteins orchestrate capsid envelopment and virion egress.

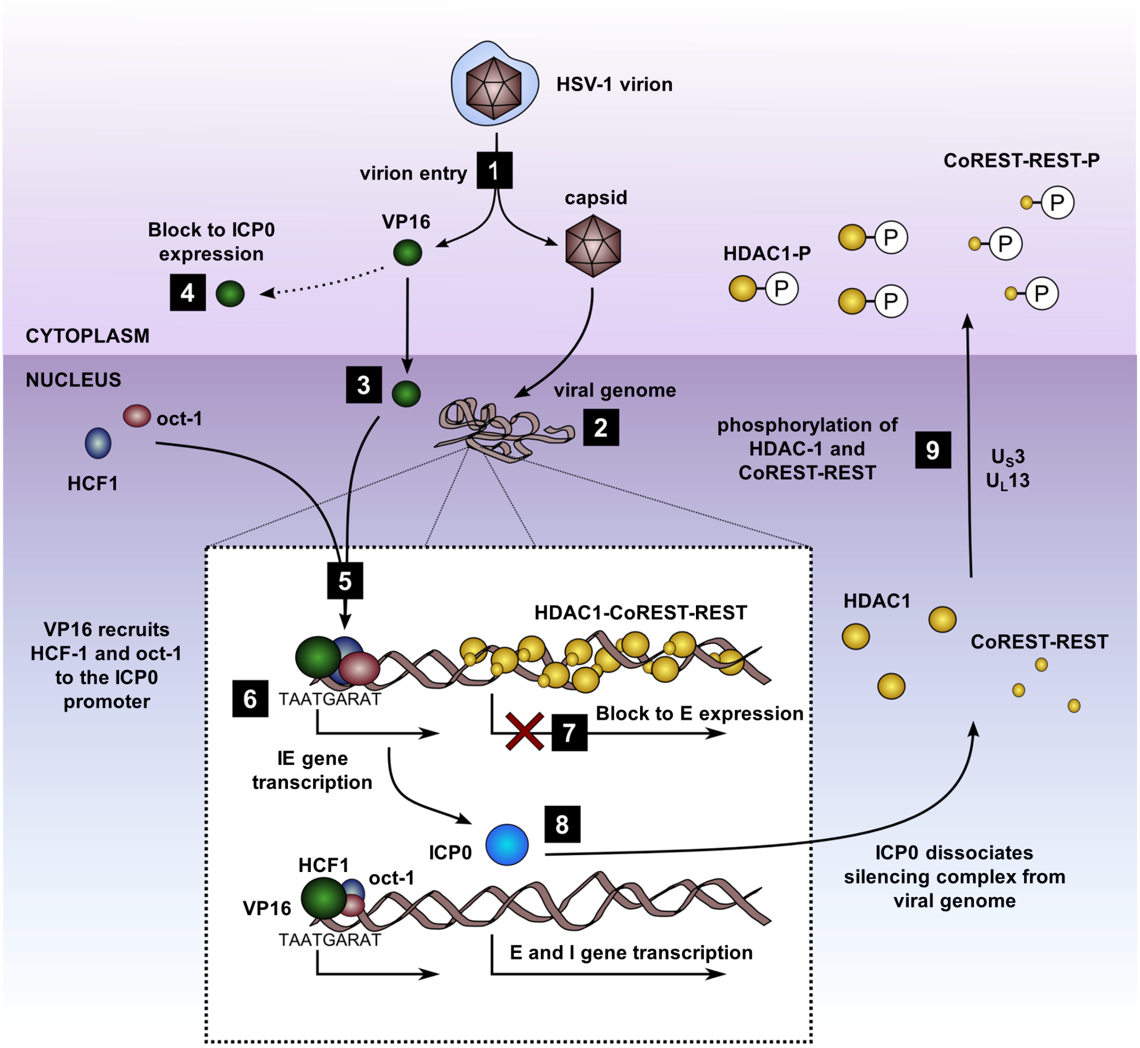

Figure 6: Blocks to productive HSV-1 infection.
[1-2] HSV-1 virions enter the cell, the viral genome uncoats and is imported into the nucleus. [3] During productive infection, the viral transcriptional transactivator VP16, brought into the cell within the virion particle, translocates to the nucleus(545). [4] In neurons supporting latent infection, VP16 is retained in the cytoplasm. This restriction provides the first block to productive viral infection, such that HSV-1 latency is established. [5] During lytic infection, VP16 in the nucleus recruits the cellular factors oct-1 and HCF1 to the IE promoters [6], and initiates IE gene transcription(546–548). HCF1 is a ubiquitous nuclear protein required for the progression through the G1 phase of the cell cycle(545). [6] The VP16-oct-1-HCF1 complex binds a specific sequence, TAATGARAT, within the viral promoter(549). [7] At this point in the viral replication cycle, E and L genes are silenced by the cellular HDAC1-CoREST-REST complex, which binds to the viral genome(550). [8] ICP0, an IE protein, promotes transcription of E and L genes by removing the HDAC1-CoREST-REST silencing complex from the viral genome(550). [9] ICP0 contains a carboxyl-terminal domain sequence homologous to a corresponding sequence near the N-terminus of CoREST. In the absence of functional ICP0, viral replication is blocked following IE gene expression and prior to DNA replication. This is referred to as quiescent infection, and can be reversed by addition of ICP0 or by activating ICP0 function. If this block is overcome, HDAC1 and CoREST-REST are phosphorylated and exported from the nucleus by the viral proteins U_S3 and U_L13(545,551). There are therefore 2 blocks to lytic replication that must be overcome during lytic replication that are overcome by initiation of IE gene expression by VP16 and removal of the HDAC1-CoREST-REST silencing complex by ICP0 [4 and 7].

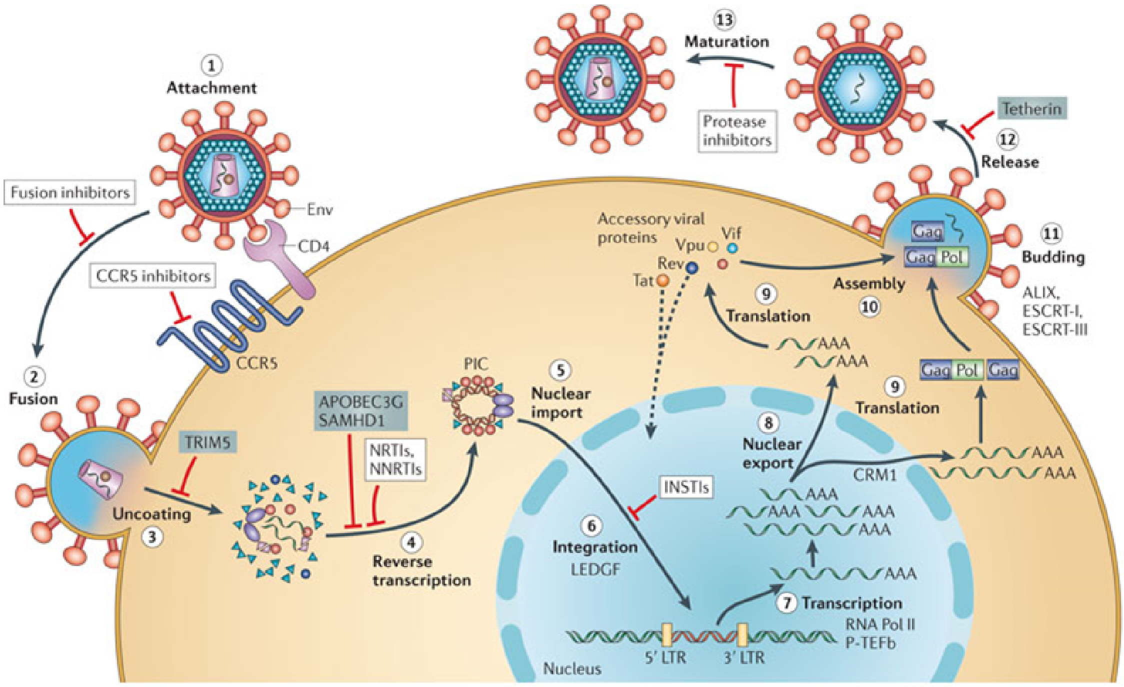

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Figure 7: HIV-1 life cycle.
This figure is taken from Engelman *et al*[254]. Following virion attachment and fusion (1,2), the viral capsid uncoats (3). The RNA genome is reverse transcribed, forming the pre-integration complex (4). The pre-integration complex is integrated into the host cell genome followed by viral transcription and translation (7, 8). Viral proteins assemble into the nascent viral capsid that buds from the cell (11-13)

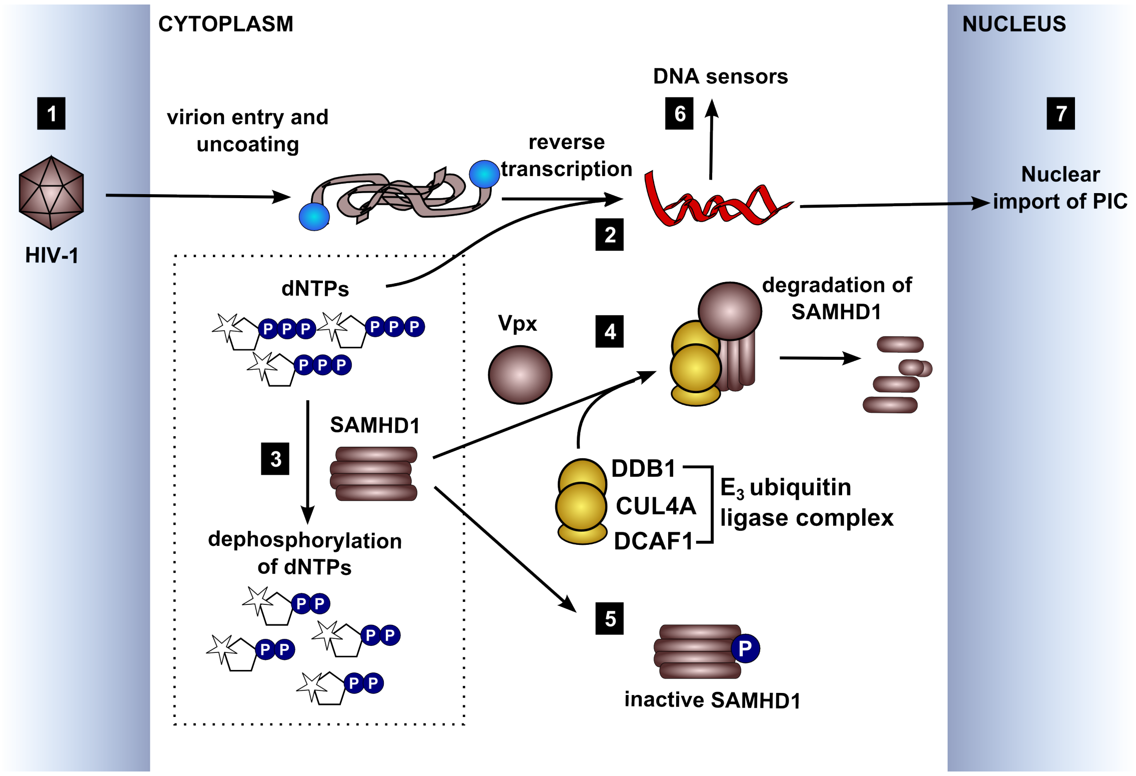

Figure 8: Vpx and SAMHD1.
[1] During entry the HIV-1 virion uncoats. [2] The dsRNA genome is reverse transcribed into the dsDNA provirus using dNTPs within the cytoplasm. [3] In non-cycling cells SAMHD1 decreases the pool of dNTPs in the cytoplasm. [4] Vpx binds SAMHD1 and promotes its degradation by recruiting the DDB1-CUL4A-DCAF1 E3 ubiquitin ligase complex. [5] SAMHD1 can also be deactivated by phosphorylation of cellular CDK1 in cycling cells. [6] HIV-1 nucleic acids in the cytoplasm can be detected by cytoplasmic PRRs. [7] The HIV-1 provirus enters the nucleus, potentially facilitated by vpx, and integrates into the host genome.

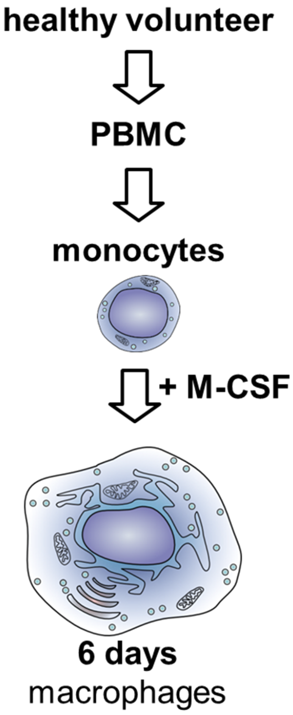

Figure 9: Monocyte derived macrophages.
120ml of blood was taken from healthy volunteers. The PBMC were extracted and the adherent monocytic cells differentiated into macrophages by incubation with M-CSF for 6 days.

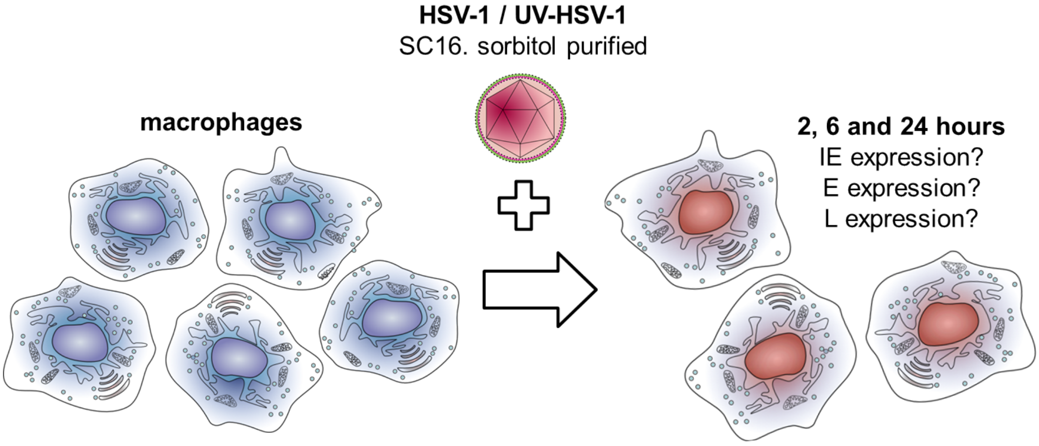

Figure 10: Experimental model to investigate HSV-1 infection of macrophages.
Monocyte derived macrophages were exposed to sorbitol purified HSV-1 or UV-HSV-1. HSV-1 gene and protein expression were assessed at various time points post infection.

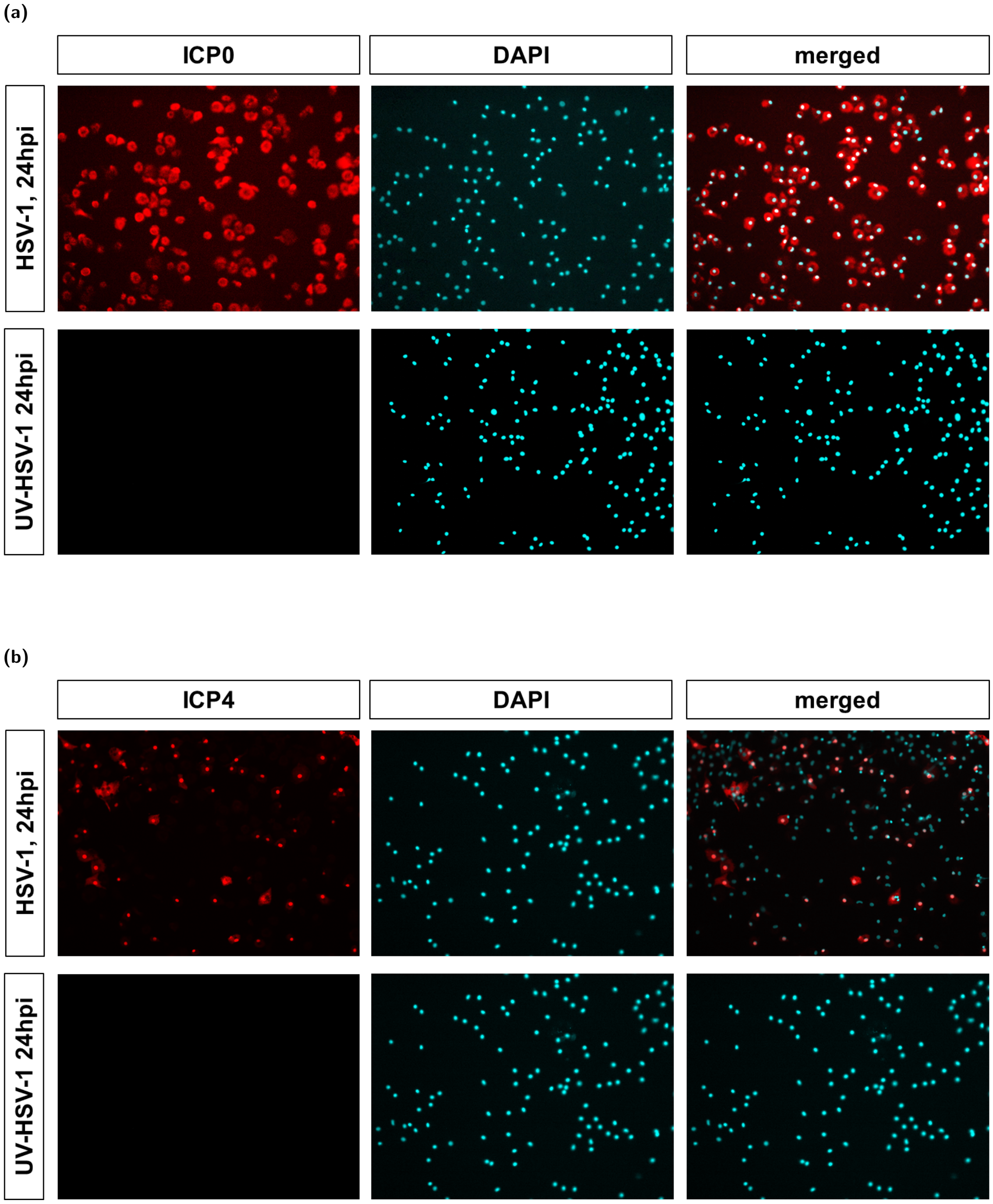

Figure 11: HSV-1 ICP0 and ICP4 protein expression in macrophages.
Macrophages were exposed to either HSV-1 or UV inactivated HSV-1. 6hpi, the cells were fixed, permeabilised and stained for ICP0 (a) or ICP4 (b) expression. Representative images are shown for macrophages exposed to 250pfu$_{vero}$/cell of HSV-1 or UV-HSV-1.

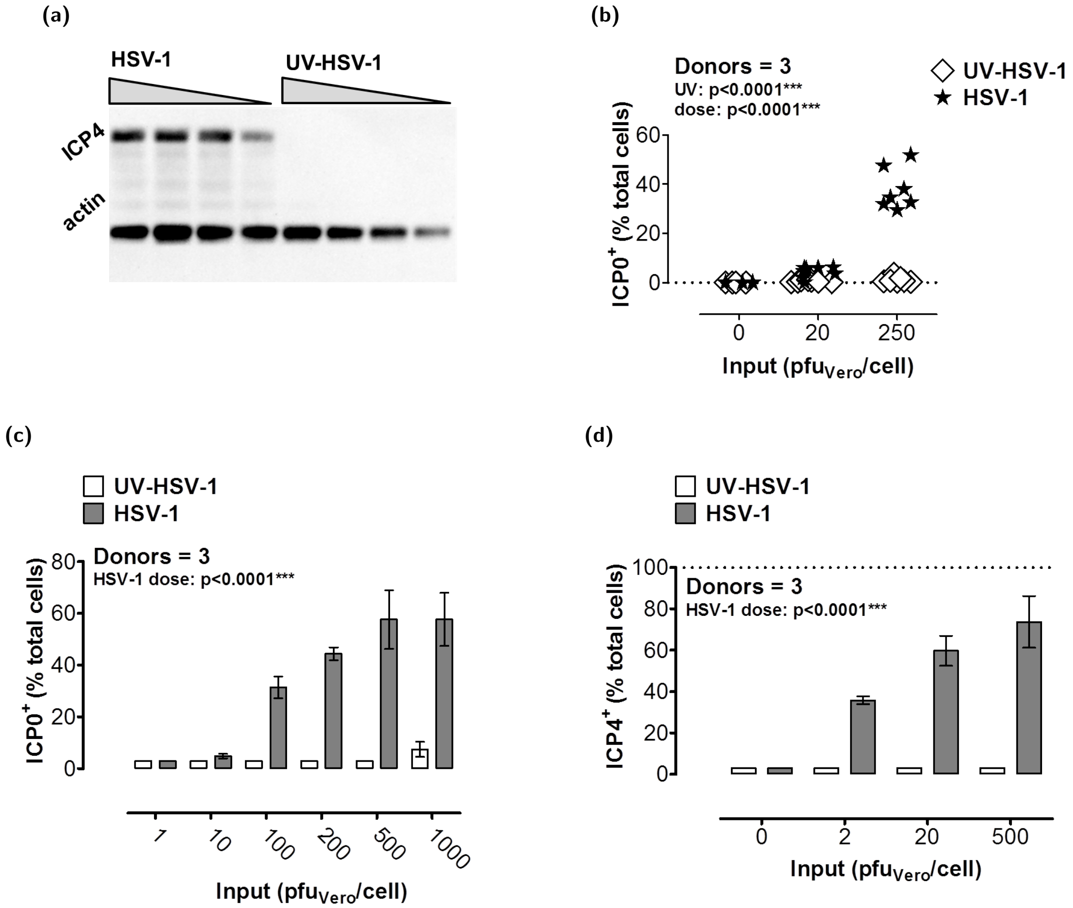

Figure 12: HSV-1 IE protein expression in macrophages.
(a): Macrophages were exposed to either HSV-1 or UV inactivated HSV-1. The dose of HSV-1 ranged from 1 to 1000 pfu_{vero}/cell. 6hpi, ICP4 expression was assessed by western blot. (b) Macrophages were infected with UV-HSV-1 or HSV-1 expressing ICP0-YFP. 6hpi, the cells were detached from the plate by 45 minutes incubation in 5% trypsin. The cells were fixed and ICP0-YFP positivity was determined using FACS. Each point on the graph represents the total macrophages from one well of a 24 well plate. (c) and (d): Macrophages were exposed to either HSV-1 or UV inactivated HSV-1. 6hpi, the cells were fixed, permeabilised and stained for ICP0. ICP0$^+$ (c) or ICP4$^+$ (d) cells were counted using Metamorph image analysis software and the number of positive cells were represented as a percentage of the total number of macrophages imaged (n 10,000) for each condition. Each bar represents the mean of 3 donors, 6 replicate wells per donor. Error bars = SEM. The 1 way ANOVA p values are shown for the effect of HSV-1 dose on the percentage of cells expressing ICP0 or ICP4.

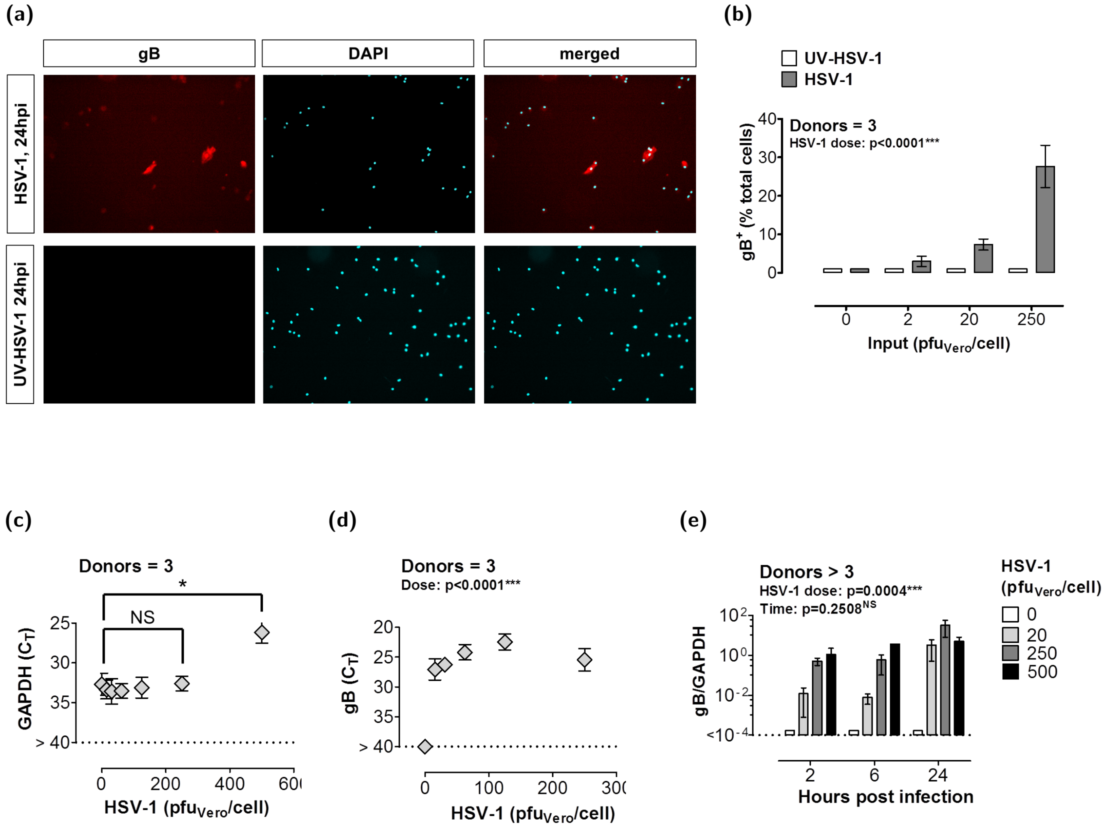

Figure 13: HSV-1 gB expression in macrophages.
(a) and (b): Macrophages were exposed to either HSV-1 or UV inactivated HSV-1. 6hpi, the cells were fixed, permeabilised and stained for gB expression. (a): Representative images are shown for macrophages exposed to 250pfu$_{vero}$/cell of HSV-1 or UV-inactivated HSV-1. (b): gB^{+} cells were counted using Metamorph image analysis software and the number of positive cells were represented as a percentage of the total number of macrophages imaged for each condition. Each bar represents the mean of 3 donors, 6 replicate wells per donor. Error bars = SEM. One way ANOVA value is shown for the effect of HSV-1 dose on the percentage of cells expressing gB. (c) to (e): Macrophages were exposed to HSV-1, total cell RNA was extracted, DNase treated and reverse transcribed into cDNA. GAPDH and HSV-1 gB expression was determined by RT-qPCR. (c): The C$_T$ values for the GAPDH expression levels. Each point represents the mean of 3 donors. Error bars = SEM. (d): The C$_T$ values for the gB expression levels. Each point represents the mean of 3 donors. Error bars = SEM. The 1 way ANOVA value is shown for the effect of HSV-1 dose on gB expression. (e): gB transcript levels were normalized to GAPDH expression. Each bar represents the mean of 3 donors. Error bars = SEM. 2 way ANOVA p values are shown for the effect of dose or time on gB expression.

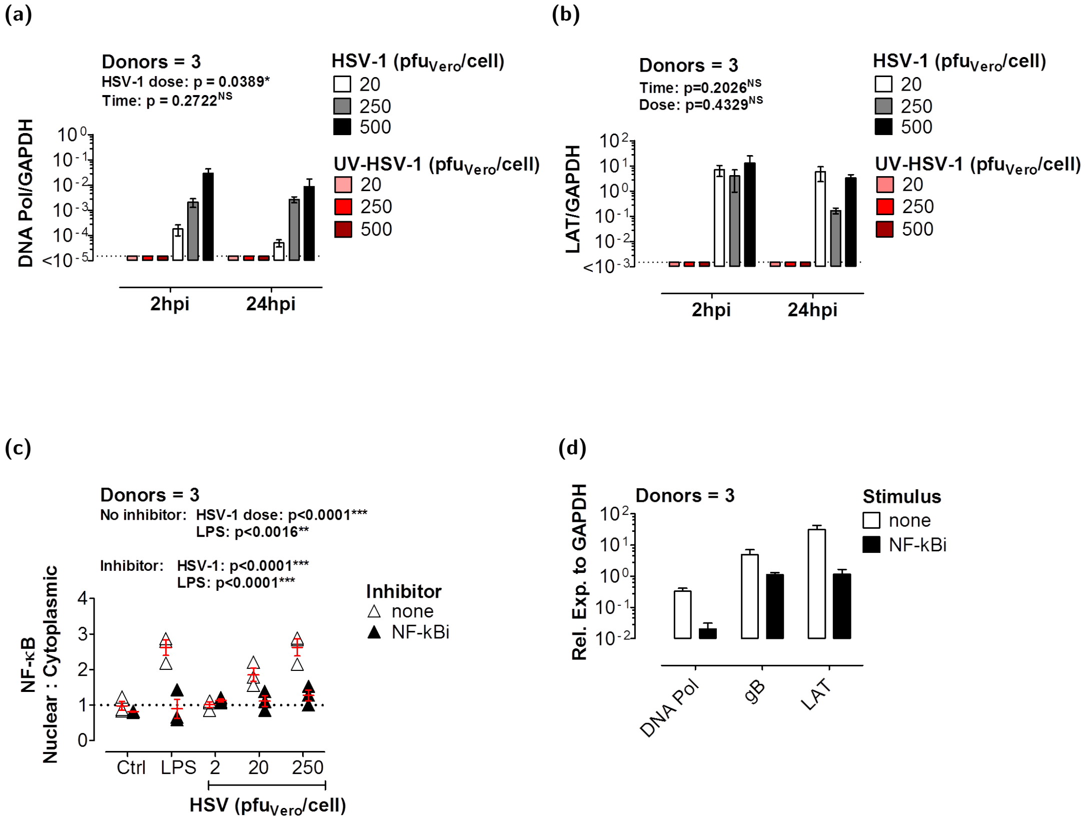

Figure 14: HSV-1 E and L gene expression in macrophages.
(a) and (b): Macrophages were exposed to HSV-1 or UV-HSV-1. 2, 6 or 24hpi, total cell RNA was extracted, DNase treated and reverse transcribed into cDNA. GAPDH and HSV-1 DNA pol (a) and HSV-1 LAT (b) expression was determined by RT-qPCR. HSV-1 transcript levels were normalized to GAPDH expression. Each bar represents the mean of 3 donors. Error bars = SEM. The 2 way ANOVA p values are shown for the effect of HSV-1 dose or time on HSV-1 E gene expression. (c): Macrophages were exposed to HSV-1 or incubated with LPS, in the presence or absence of an NF-κB inhibitor. After 2 hours, the cells were fixed, permeabilised and stained for NF-κB. Image analysis software was used to calculate the ratio of cytoplasmic to nuclear NF-κB in each imaged cell. Each data point represents the mean of 6 replicate wells per donor. The line represents the mean of all three donors. Error bars = SEM. 2 way ANOVA p values are shown for the effect of HSV-1 or LPS on NF-κB activation, in the presence and absence of the inhibitor. (d): Macrophages were infected with HSV-1 in the presence or absence of an NF-κB inhibitor. 24hpi, total cell RNA was extracted, DNase treated and reverse transcribed into cDNA. GAPDH, HSV-1 DNA Pol and LAT expression were determined by RT-qPCR. HSV-1 transcript levels were normalized to GAPDH expression. Each bar represents the mean of 3 donors. Error bars = SEM.

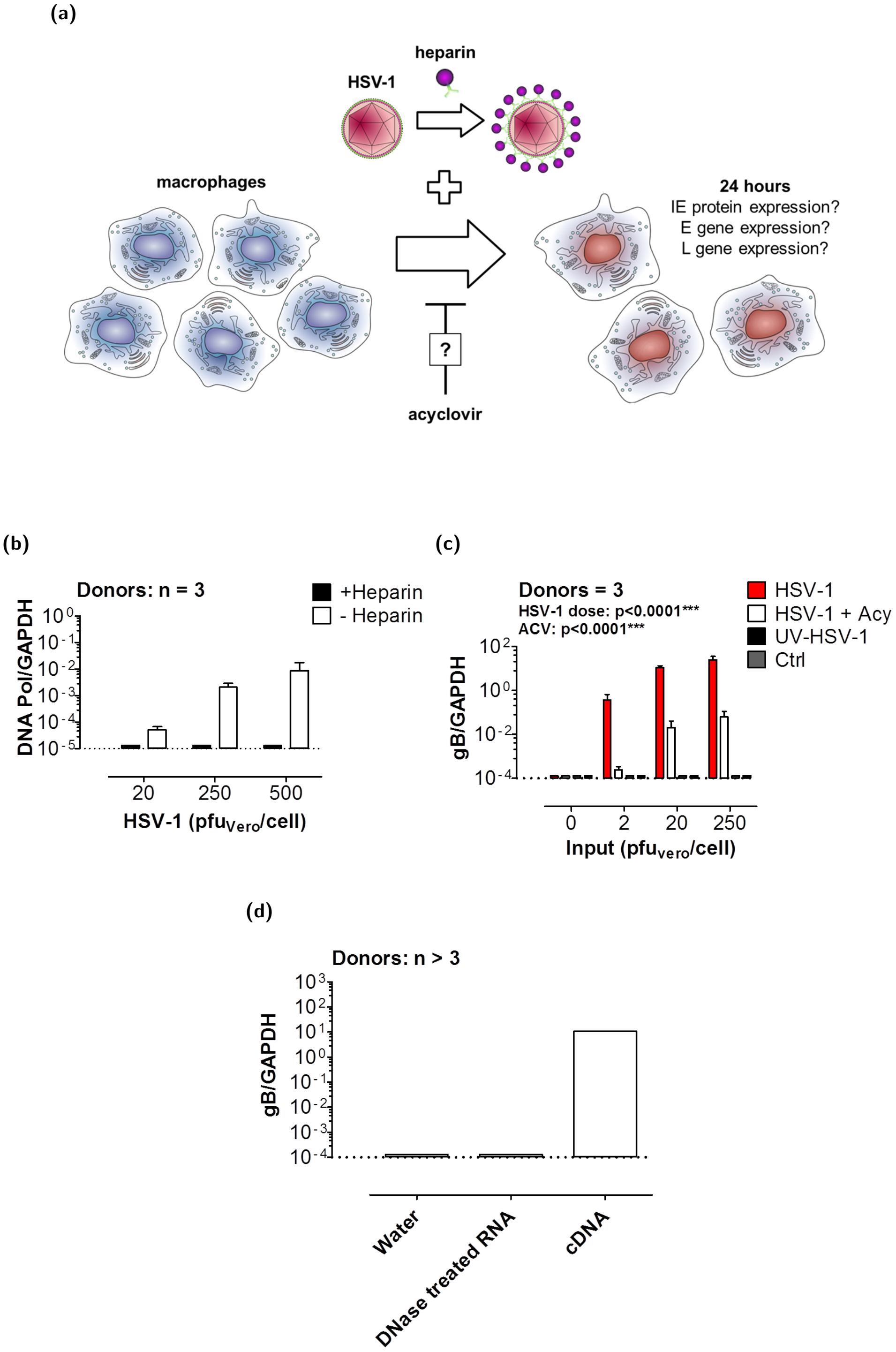

Figure 15: Inhibiting HSV-1 infection of macrophages.
(a): Macrophages were exposed to HSV-1 treated with soluble heparin or infected with HSV-1 in the presence of acyclovir. (b): Macrophages were exposed to HSV-1 or HSV-1 that had been incubated with heparin. 24hpi, HSV-1 DNA Pol transcript levels were assessed by RT-qPCR and normalised to GAPDH expression. Each bar represents the mean of 3 donors. (c): Macrophages were exposed to UV-HSV-1 or HSV-1 in the presence or absence of acyclovir. 24hpi, gB transcript levels were assessed by RT-qPCR and normalised to GAPDH expression. Each bar represents the mean of 3 donors. Error bars = SEM. 2 way ANOVA p values are shown for the effect of dose or acyclovir on gB expression. (d): The efficiency of the DNase treatment was assessed by performing qPCR for gB transcript on the DNase treated RNA samples. Each bar represents the mean of >20 donors.

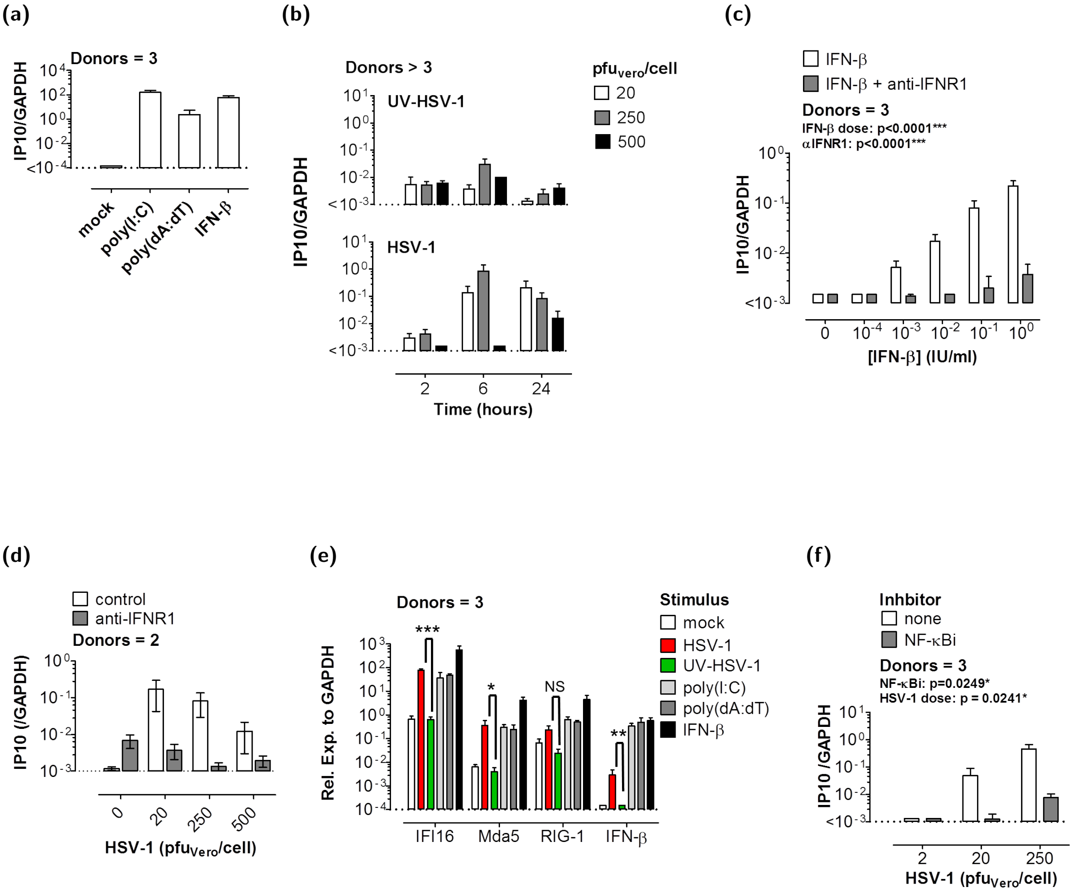

Figure 16: HSV-1 induces a type I IFN response in macrophages.
(a): Macrophages were stimulated with either IFN-β, poly(I:C) or poly(dA:dT) for 6 hours. Cellular RNA was harvested, purified and reverse transcribed into cDNA. RT-qPCR for IP10 and GAPDH transcript was performed, and IP10 expression normalised to GAPDH expression. Each bar represents the mean of 3 donors. Error bars = SEM. (b): Macrophages were infected with HSV-1 or exposed to UV-HSV-1. 2, 6 and 24hpi viral and cellular RNA was harvested, purified and reverse transcribed into cDNA. RT-qPCR was performed for IP10 and GAPDH expression. IP10 transcript levels were normalised to GAPDH expression. Each bar represents the mean of >3 donors. Error bars = SEM. (c) and (d): Macrophages were stimulated with recombinant IFN-β (c) or infected with HSV-1 (d), in the presence or absence of an IFNR blocking antibody. After 6 (c) or 24 (d) hours, cellular RNA was harvested, purified and reverse transcribed into cDNA. A RT-qPCR for IP10 and GAPDH transcript was performed, and IP10 expression normalised to GAPDH expression. Each bar represents the mean of 3 donors. Error bars = SEM. 2 way ANOVA p values are shown for the effect of IFN-β dose and IFNR blocking antibody on IP10 expression. (e): Macrophages were infected with HSV-1. 24hpi viral and cellular RNA was harvested, purified and reverse transcribed into cDNA. RT-qPCR was performed for IFI16, Mda5, RIG-I, IFN-β and GAPDH expression. ISG transcript levels were normalised to GAPDH expression. Each bar represents the mean of 3 donors. Error bars = SEM. t-tests were performed for the difference between ISG expression levels following exposure to either HSV-1 or UV-HSV-1. (f): Macrophages were infected with HSV-1 in the presence or absence of an NF-κB signalling inhibitor. 24hpi, viral and cellular RNA was harvested, purified and reverse transcribed into cDNA. RT-qPCR was performed for IP10 and GAPDH expression. IP10 transcript levels were normalised to GAPDH expression. Each bar represents the mean of 3 donors. Error bars = SEM. 2 way ANOVA p values are shown for the effect of HSV-1 dose on the NF-κB inhibitor on IP10 upregulation.

(a)

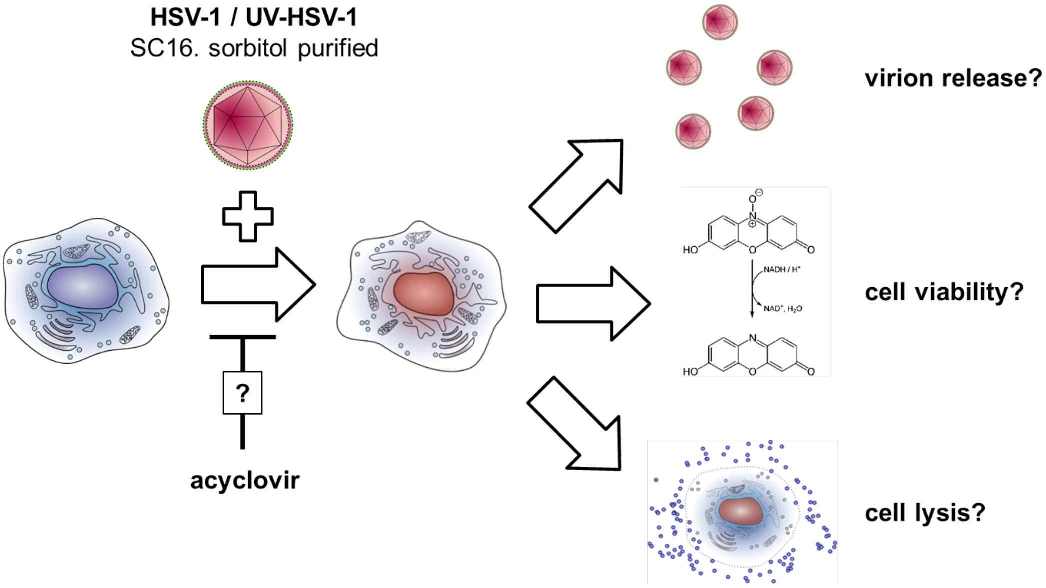

Figure 17: Assessing cell death.
HSV-1 macrophages were infected with sorbitol purified HSV-1 or UV-HSV-1, in the presence or absence of acyclovir. At various time points, virion release, cell viability and cell lysis was assessed. Cell viability was determined by measuring the reduction capacity of a population of cells. Cell lysis was determined by assessing LDH release.

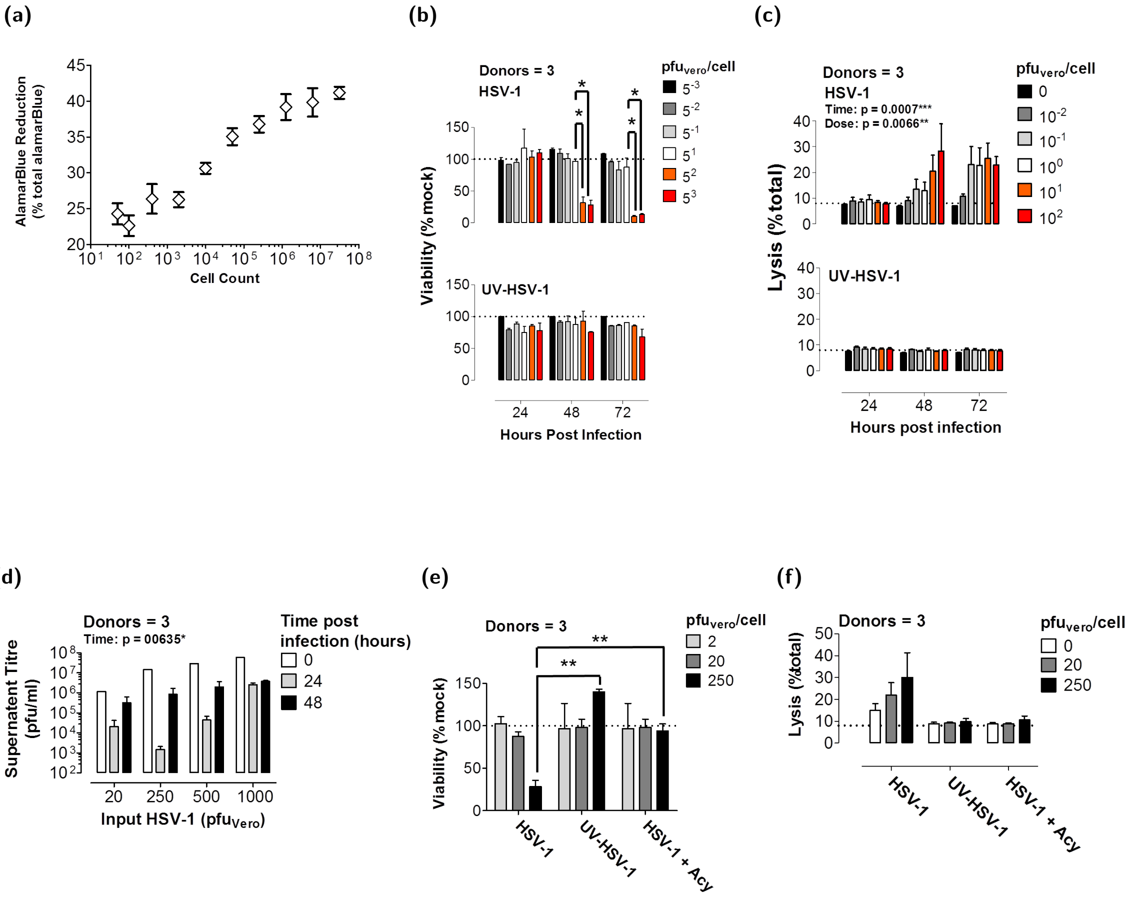

Figure 18: HSV-1 productively infects macrophages.

(a) and (b): The alamarBlue assay was used to assess the cell viability of populations of 293T cells of known cell number (a) or macrophages were exposed to HSV-1 or UV-HSV-1, 24hpi, 48hpi or 72hpi (b). Each point represents the mean of 3 experiments (a). Each bar represent the mean of 3 donors (b). Error bars = SEM. t-tests were performed to assess the significance of the difference between viability of macrophages exposed to 5 and 50 or 500pfu$_{v}ero/$cell. (c): Macrophages were infected with HSV-1 or UV-HSV-1. 24, 48 and 72hpi, the supernatents were collected and LDH levels assessed by ELISA. Cell lysis is represented as the LDH released from a sample as a percentage of the LDH that would be released during lysis of the total macrophage population. 2 way ANOVA p values are shown for the effect of HSV-1 dose and time on macrophage death. (d): Macrophages were infected with various doses of HSV-1. 1hpi, the cells were washed with PBS and the cell media replaced. 24hpi and 48hpi, the cell supernatents were collected and replaced with media. The supernatents were frozen, thawed at a later date and titred by a vero cell plaque assay. Time 0 represents the input dose of HSV-1. Each bar represents the mean of 3 donors, 3 replicates per donor. The 1 way ANOVA p-value is shown for the effect of HSV-1 dose on virion production. (e) and (f): Macrophages were infected with HSV-1, exposed to HSV-1 in the presence of acyclovir or exposed to UV-HSV-1. 48hpi, the cell viability was assessed by the alamarBlue assay (e), or the supernatents collected to assess cell lysis by LDH ELISA (f). Each bar represents the mean of 3 donors. Error bars = SEM. t-tests were performed.

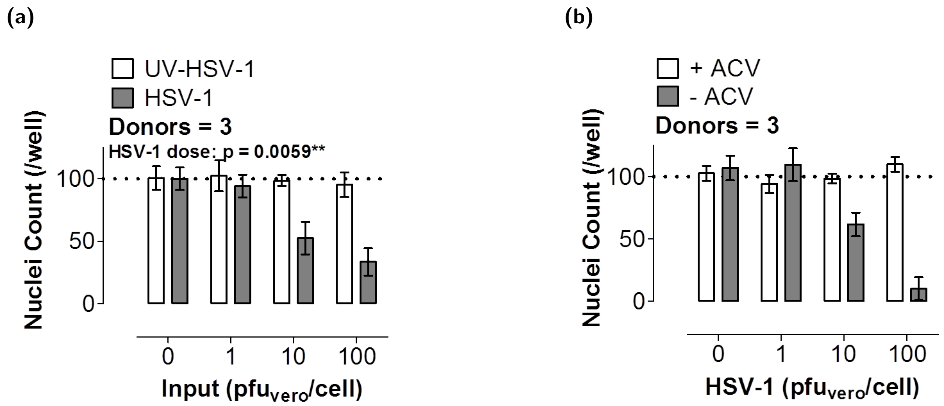

Figure 19: Nuclei counting.
Macrophages were infected with various doses of HSV-1, exposed to UV-HSV-1, (a), or infected with HSV-1 in the presence or absence of acyclovir (b). 24hpi, the cells were fixed, stained with DAPI and imaged using an automated fluorescent microscope. For each well of the plate, 23 non-overlapping images were taken. The number of nuclei per image were counted using image analysis software and added to give the total number of nuclei per well. The total number of nuclei in an infected well of macrophages is represented as a percentage of the total number of macrophages in a mock infected well. Each point represents the mean of 6 wells, from 1 donor (a). Each bar represents the mean of 3 donors. Error bars = SEM. The 1 way ANOVA p value shown for the effect of HSV-1 dose on nuclei count.

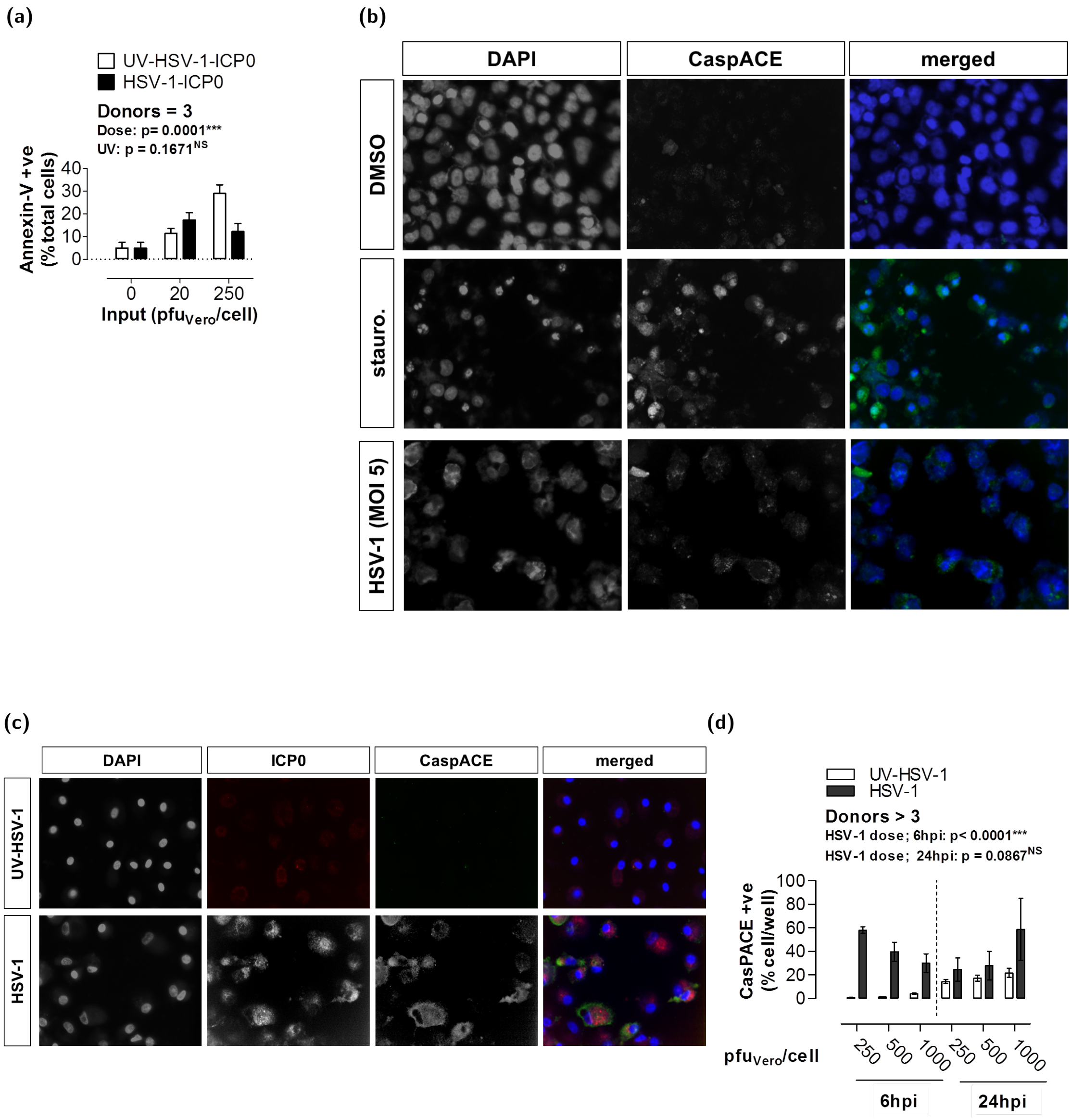

Figure 20: Caspase activation and membrane permeability in HSV-1 infected macrophages.
(a): Macrophages were infected with UV-HSV-1 or HSV-1 expressing ICP0-YFP. 6hpi the cells were detached from the plate by incubation for 45 minutes in 5% trypsin. The cells were fixed, stained for Annexin V and Annexin V positivity determined via FACS. Each bar represents the mean of 2 donors, 3 replicates per donor. Error bars = SEM. 2 way ANOVA p values are shown for the effect of HSV-1 dose or UV inactivation on Annexin staining. (b): HeLa cells were infected with HSV-1 or incubated with media containing either staurosporine or a DMSO loading control. After 24 hours, the cells were incubated with media containing the fluorescently labelled caspase inhibitor CaspACE-FITC for 20 minutes. The cells were then fixed, permeabilised, stained with DAPI and imaged. (c) and (d): Macrophages were infected with HSV-1. At 6 or 24hpi, the cells were incubated with media containing the fluorescently labelled caspase inhibitor CaspACE-FITC for 20 minutes. The cells were then fixed, permeabilised, stained with DAPI, stained for ICP0 expression and imaged. (d): CaspACE^{+} cells were scored using Metamorph image analysis software and the number of CaspACE^{+} cells was represented as a percentage of the total number of macrophages imaged for each condition. Each bar represents the mean of 3 donors, 12 replicate wells per donor. Error bars = SEM. The 1 way ANOVA p values are shown for the effect of HSV-1 dose on the precentage of caspACE positive cells at 6 and 24hpi.

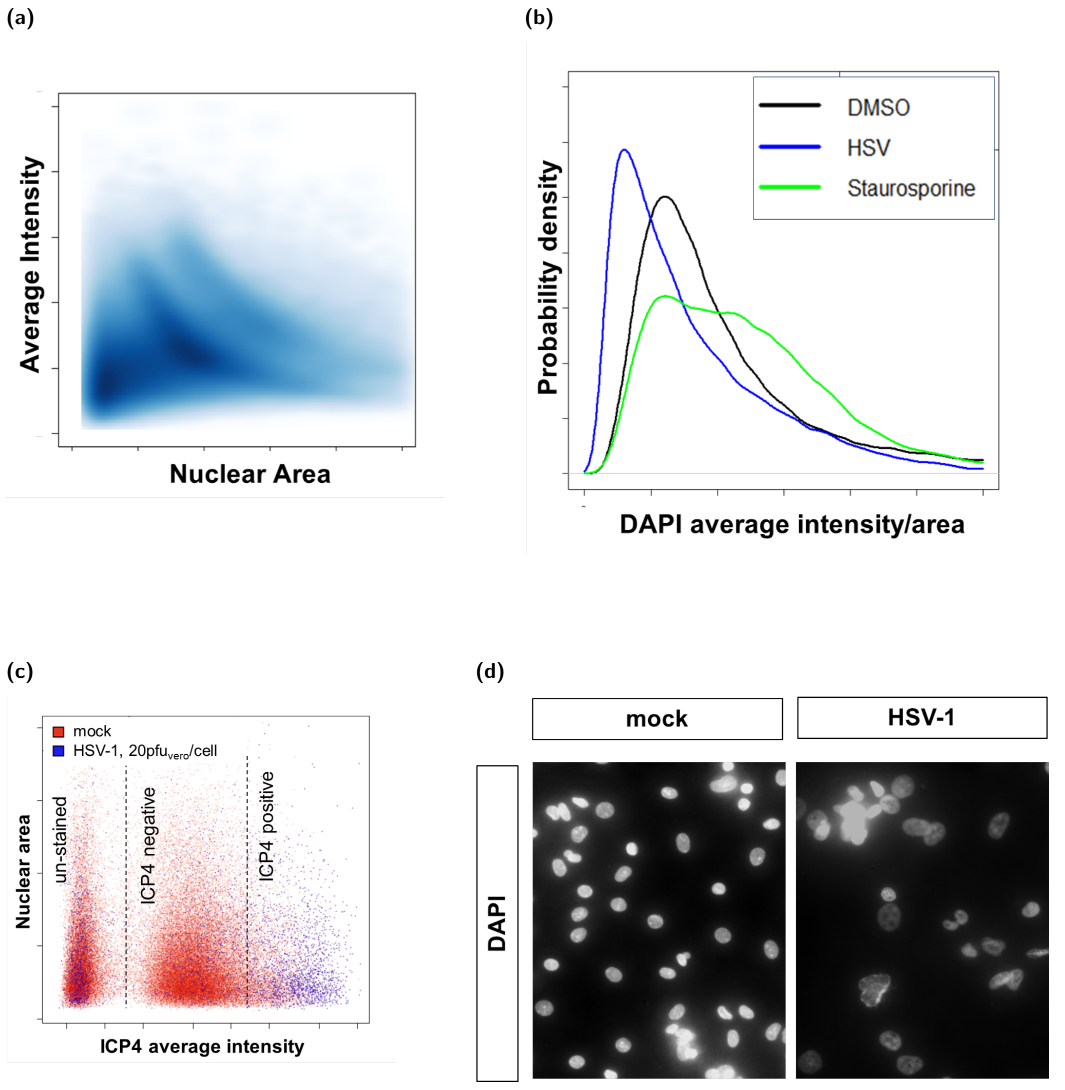

Figure 21: Analysis of nuclear morphology.
(a) and (b): HeLa cells were infected with HSV-1 or incubated with media containing either staurosporine or a DMSO loading control. After 6 hours, the cells were then fixed, permeabilised, stained with DAPI and imaged. Image analysis software was used to score the area and intensity of each nucleus (a). The scales on this plot are arbitrary. The probability density of the average intensity and area of the nuclei were plotted using R (b). (c): Macrophages were infected with HSV-1. After 24 hours, the cells were then fixed, permeabilised, stained with DAPI and for ICP4 expression and imaged. Image analysis software was used to score each nucleus for average ICP4 expression and nuclear area. (d): Macrophages were infected with HSV-1 or incubated with media containing either staurosporine or a DMSO loading control. After 24 hours, the cells were then fixed, permeabilised, stained with DAPI and imaged.

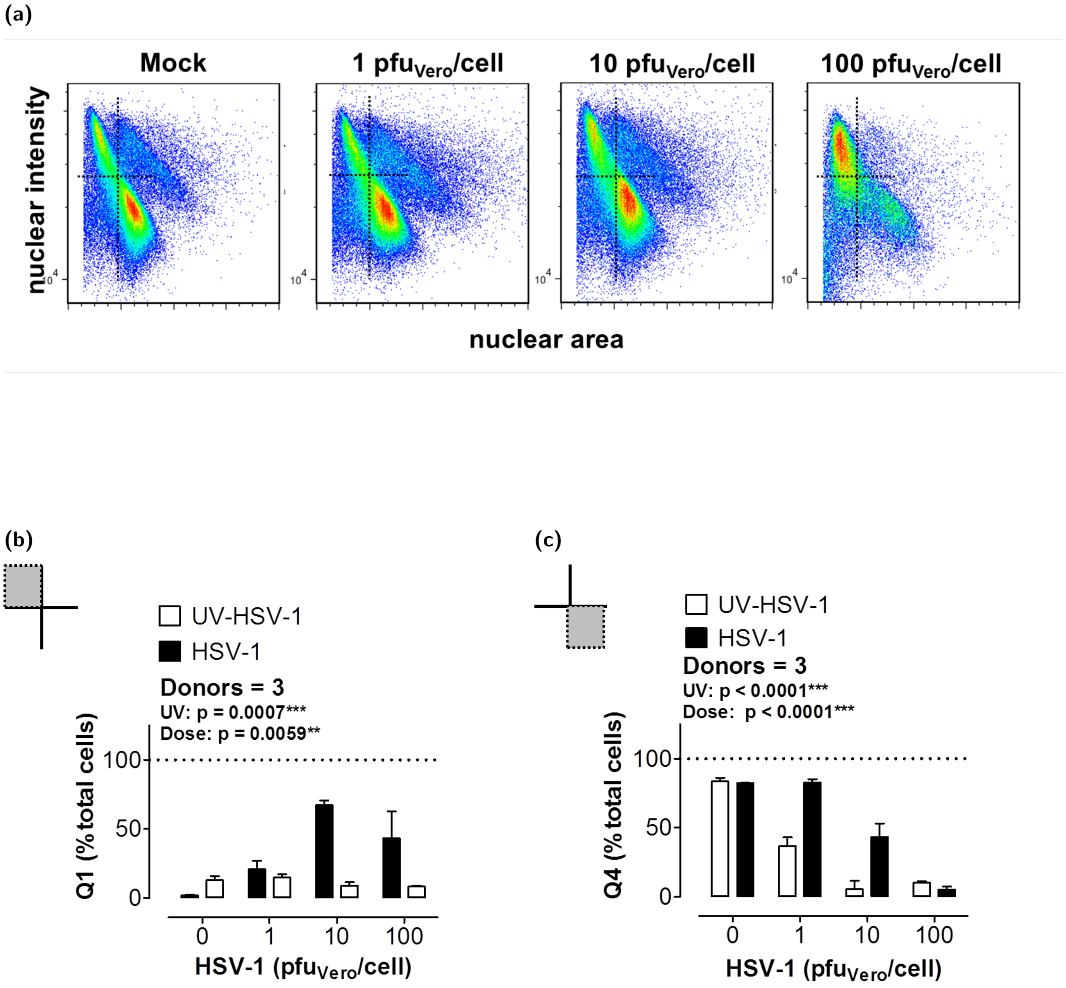

Figure 22: Analysis of nuclear morphology in HSV-1 infected macrophages.
Macrophages were infected with HSV-1. 24hpi, the cells were fixed, permeabilised, stained with DAPI and imaged. Image analysis software was used to score the area and intensity of each nucleus. These values were expressed as a density plot using flowJo FACS analysis software (a). The percentage of nuclei in the top left (b) and bottom left (c) quadrants were determined. 2 way ANOVA p values are shown for the effect of UV inactivation of HSV-1 and dose on HSV-1 dependent nuclear morphology. Each bar represents the mean of 3 donors. Error bars = SEM.

(a)

Pathway	**Inhibitor**	**Action**
pyroptosis	Z-YVAD-FMK	caspase-1 inhibitor
apoptosis	Z-VAD-FMK	pan-caspase inhibitor
necroptosis	nec-1	RIP1 inhibitor

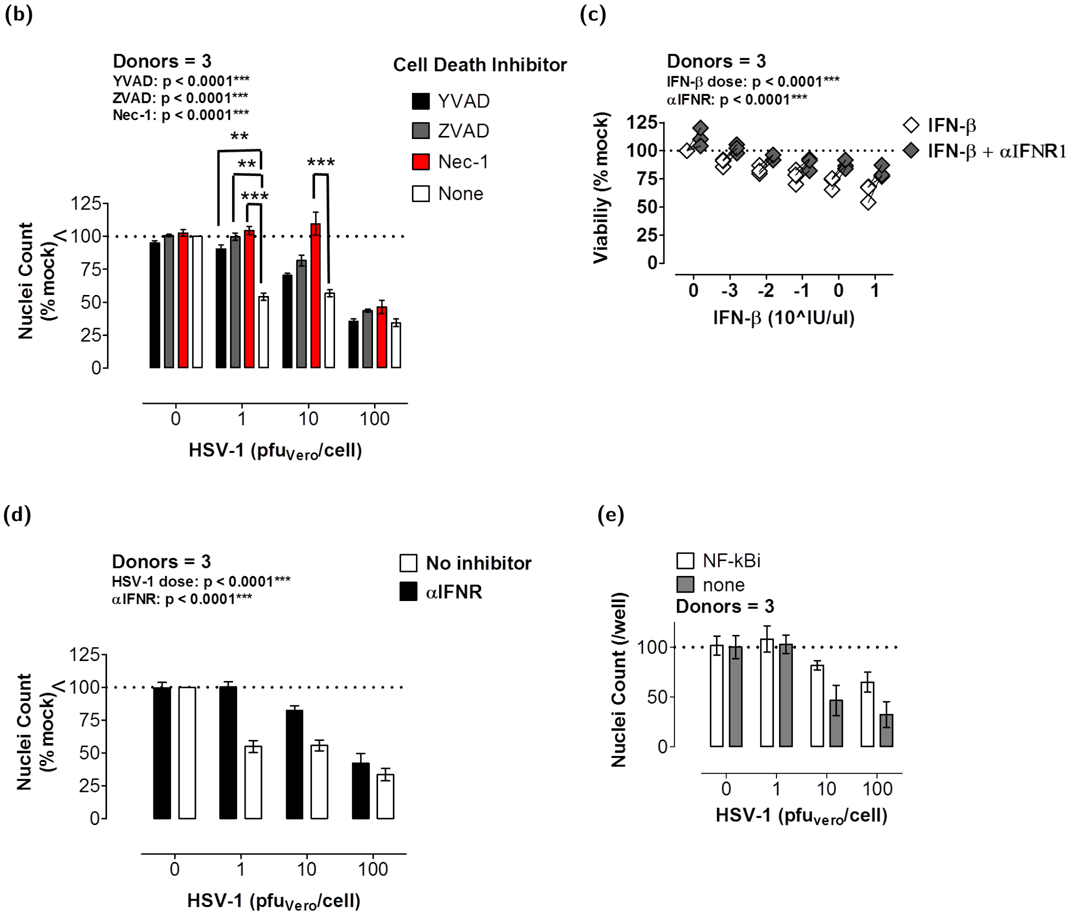

Figure 23: The mechanism of HSV-1 dependent cell death.
(a): Macrophages can die by apoptosis, pyroptosis or necroptosis. Apoptosis can be inhibited with a pan-caspase inhibitor, Z-VAD. Pyroptosis can be inhibited with Y-VAD, a caspase-1 inhibitor. Necroptosis can be inhibited with necrostatin-1 (nec-1). (b) and (d): Macrophages were infected with various doses of HSV-1 in the presence of YVAD, ZVAD, nec-1 (b), an IFNR blocking antibody (d) or no inhibitor. 24hpi, the cells were fixed, stained with DAPI and imaged using an automated fluorescent microscope. For each well of the plate, 23 non-overlapping images were taken. The number of nuclei per image were counted using image analysis software and added to give the total number of nuclei per well. The total number of nuclei in an infected well of macrophages is represented as a percentage of the total number of macrophages in a mock infected well. Each bar represents the mean of 3 donors. Error bars = SEM. 2 way ANOVA p values are shown for the effect of each inhibitor or HSV-1 dose on nuclei count. (c): Macrophages were exposed to various concentrations of recombinant IFNβ for 24 hours, in the presence or absence of an IFNR blocking antibody. Macrophage viability was assessed by the alamarBlue viability assay, and expressed as a percentage of mock stimulated cells in the presence or absence of the IFNR blocking antibody. Each point represents the mean of 3 replicates from 1 donor. Error bars = SEM. 2 way ANOVA p values are shown for the effect of IFN-β or the IFNR antibody on nuclei count. (e): Macrophages were infected with HSV-1 in the presence or absence of an NF-κB inhibitor. 24hpi, the cells were fixed, stained with DAPI and imaged using an automated fluorescent microscope. For each well of the plate, 23 non-overlapping images were taken. The number of nuclei per image were counted using image analysis software and added to give the total number of nuclei per well. The total number of nuclei in an infected well of macrophages is represented as a percentage of the total number of macrophages in a mock infected well. Each bar represents the mean of 3 donors.

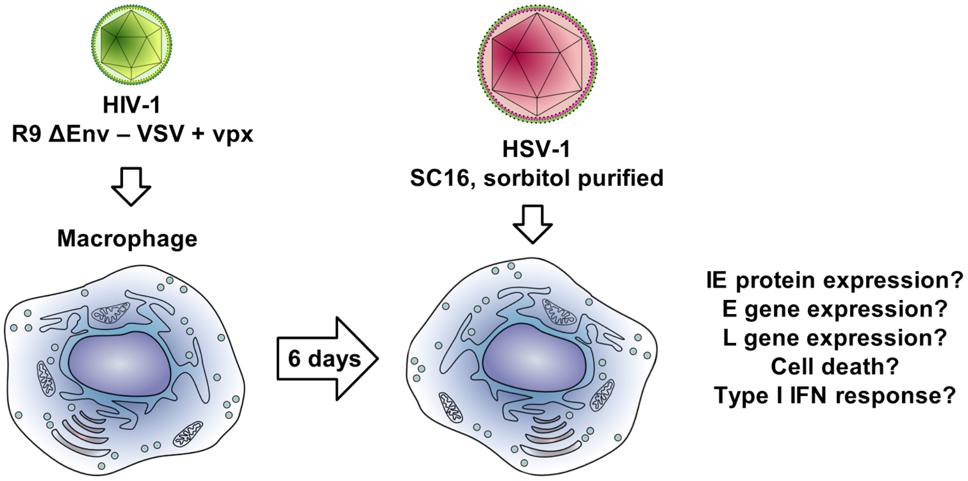

Figure 24: An experimental model to investigate HIV-1/HSV-1 co-infection.
Macrophages were infected with single round HIV-1 and VSV pseudo-typed Vpx 6 days post isolation. After 6 days, the macrophages were infected with HSV-1. 24hpi, HSV-1 gene expression, HSV-1 dependent cell death and ISG up-regulation were assessed.

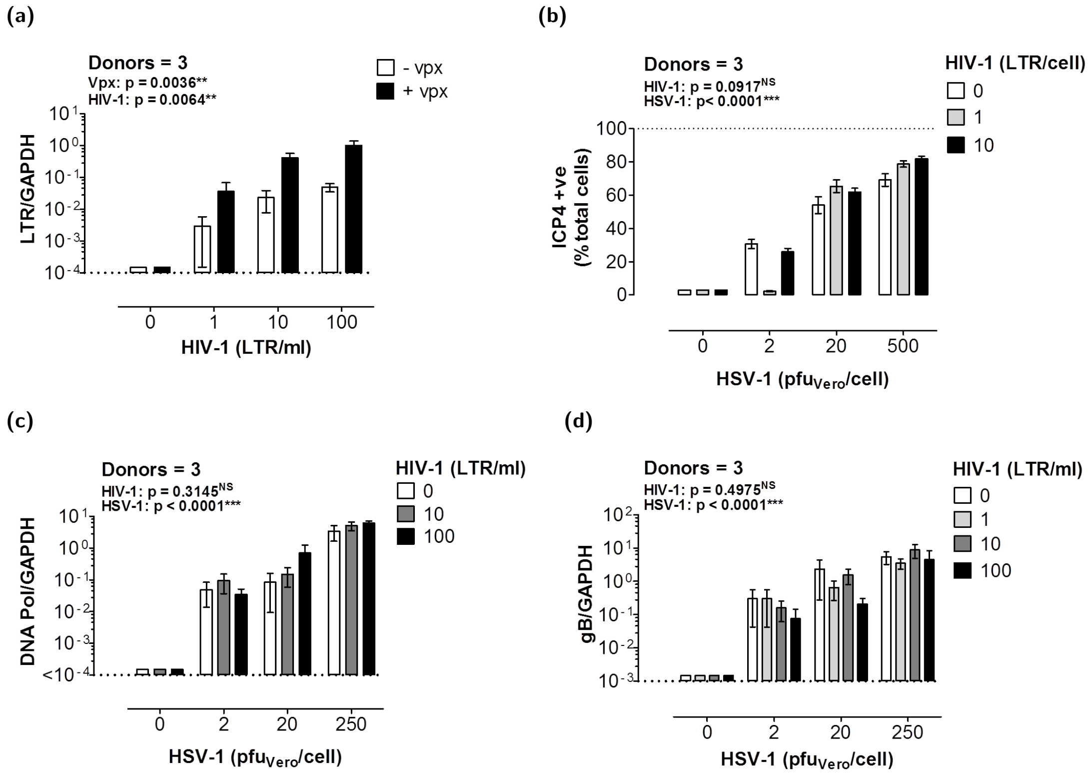

Figure 25: The effect of HIV-1 on HSV-1 gene expression.
Macrophages were infected with single-round R9 BaL ∆env HIV-1. 6dpi, the macrophages were super-infected with HSV-1. (a), (c) and (d): 24 hours after infection with HSV-1, total cellular RNA was extracted, DNase treated and reverse-transcribed into cDNA. GAPDH and HIV-1 LTR (a), HSV-1 DNA Pol (c) and HSV-1 gB (d) expression was assessed by RT-qPCR. Viral levels were normalised to GAPDH expression. (b): Co-infected macrophages were fixed, permeabilsed and stained for ICP4. ICP4$^+$ cells were scored using Metamorph image analysis software and the number of ICP4$^+$ cells was represented as a percentage of the total number of macrophages imaged for each condition. In all the graphs, each bar represents the mean of at least 3 donors. Error bars = SEM. 2 way p ANOVA values are shown for the effect of HSV-1 and HIV-1, with the Vpx control excluded if necessary.

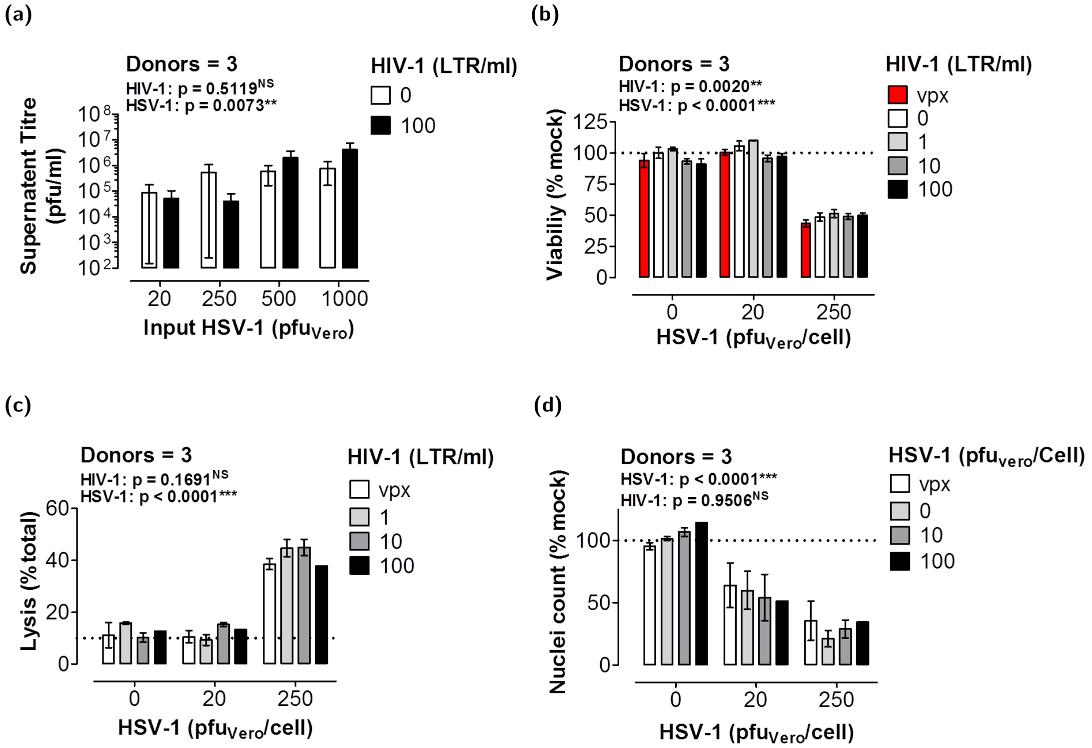

Figure 26: The effect of HIV-1 on HSV-1 productive infection.
Macrophages were infected with single-round HIV-1. 6dpi, the macrophages were super-infected with HSV-1. (a): 48hpi the supernatent was collected and assessed for the presence of nascent virions by plaque assay on vero cells. (b): 48hpi, the cells were assessed for viability by the AlamarBlue assay. Viability is represented as a percentage of the viability of mock infected cells. (c): 48hpi, the supernatents were collected and LDH levels assessed by ELISA. Cell lysis is represented as the LDH released from a sample as a percentage of the LDH that would be released during lysis of the total macrophage population. (d): The nuclei in each condition were counted using Metamorph image analysis software. The number of nuclei are represented as a percentage of the number of nuclei in the mock infected macrophage cultures. In all of the graphs, each bar represents the mean of at least 3 donors. Error bars = SEM. 2 way ANOVA p values are shown for the effect of HSV-1 and HIV-1, with the Vpx control excluded if necessary.

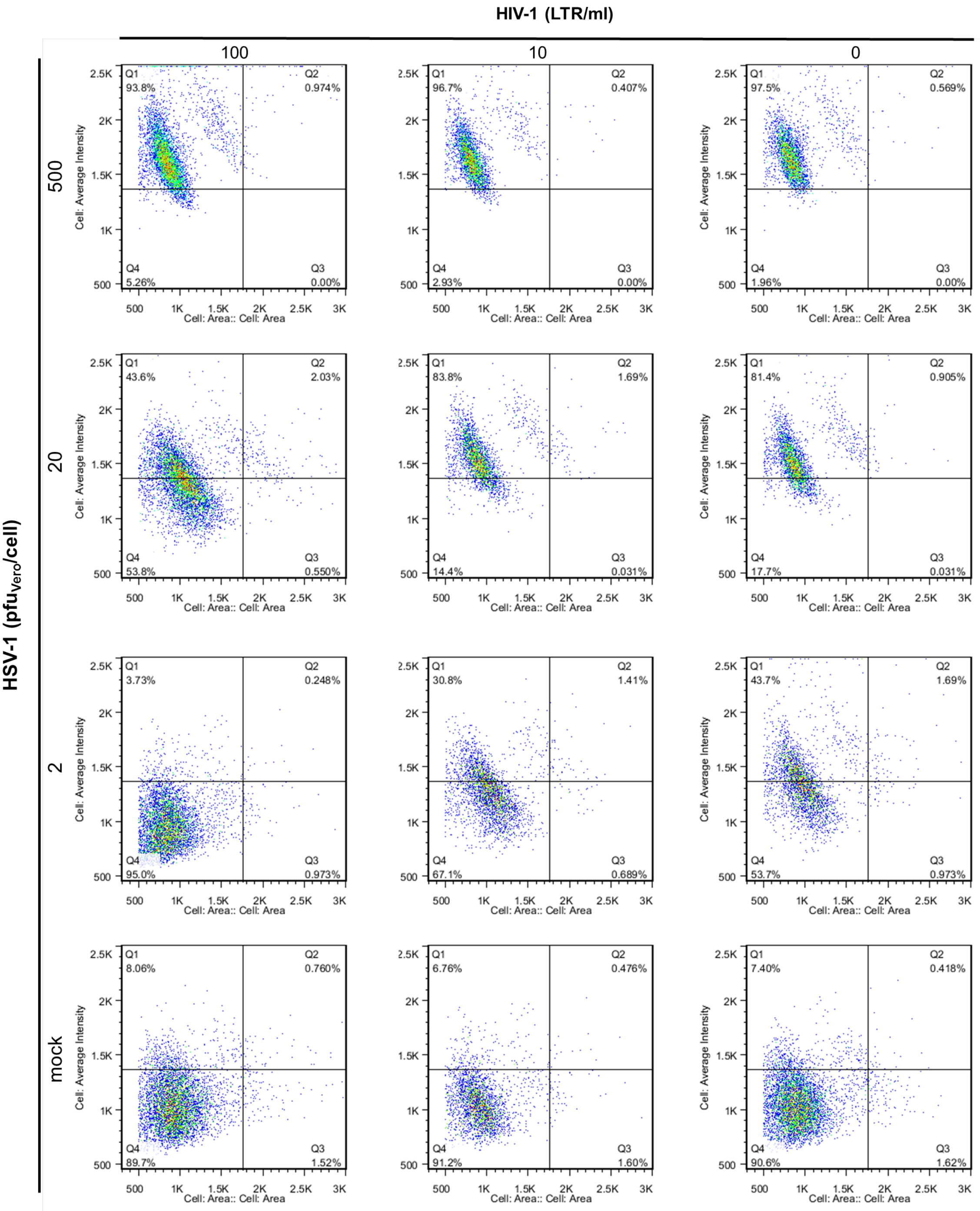

Figure 27: The effect of HIV-1 on HSV-1 dependent changes in nuclear morphology.
Macrophages were infected with single-round HIV-1 with Vpx, or Vpx only. 6dpi, the macrophages were super-infected with various doses of HSV-1. 24 hours after infection with HSV-1, the macrophages were fixed, stained with DAPI and imaged. The area and average intensity of each imaged nucleus was recorded using Metamorph image analysis software. These values were represented on density scatter plots using FlowJo.

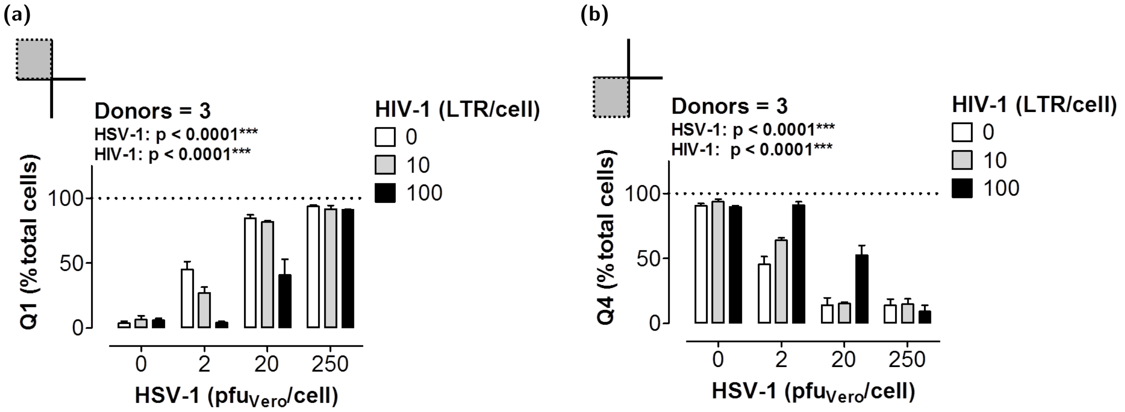

Figure 28: Summary of the effect of HIV-1 on HSV-1 dependent changes in nuclear morphology. Macrophages were infected with single-round HIV-1 with Vpx, or Vpx only. 6dpi, the macrophages were super-infected with various doses of HSV-1. 24 hours after infection with HSV-1, the macrophages were fixed, stained with DAPI and imaged. The area and average intensity of each imaged nucleus was recorded using Metamorph image analysis software. These values were represented on density scatter plots using FlowJo. The percentage of cells in the top left (b) and bottom left (a) quadrants are represented. In each graph, each bar represents the mean of at least 3 donors. Error bars = SEM. 2 way ANOVA p values are shown for the effect of HSV-1 and HIV-1.

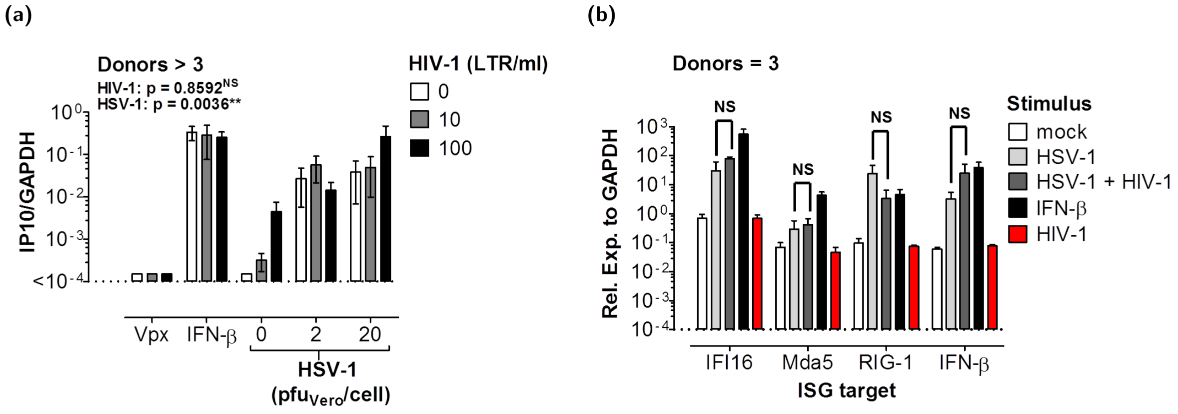

Figure 29: The effect of HIV-1 on the type I IFN response to HSV-1 infection.
Macrophages were infected with single round HIV-1. 6dpi, the macrophages were super-infected with HSV-1. 24 hours after infection with HSV-1, total cellular RNA was extracted, DNase treated and reverse-transcribed into cDNA. GAPDH and IP10 (a) and various ISGs (b) expression levels were assessed by RT-qPCR. ISG transcript levels were normalised to GAPDH expression. In each graph, each bar represents the mean of at least 3 donors. Error bars = SEM. 2 way ANOVA p values are shown for the effect of HSV-1 and HIV-1.

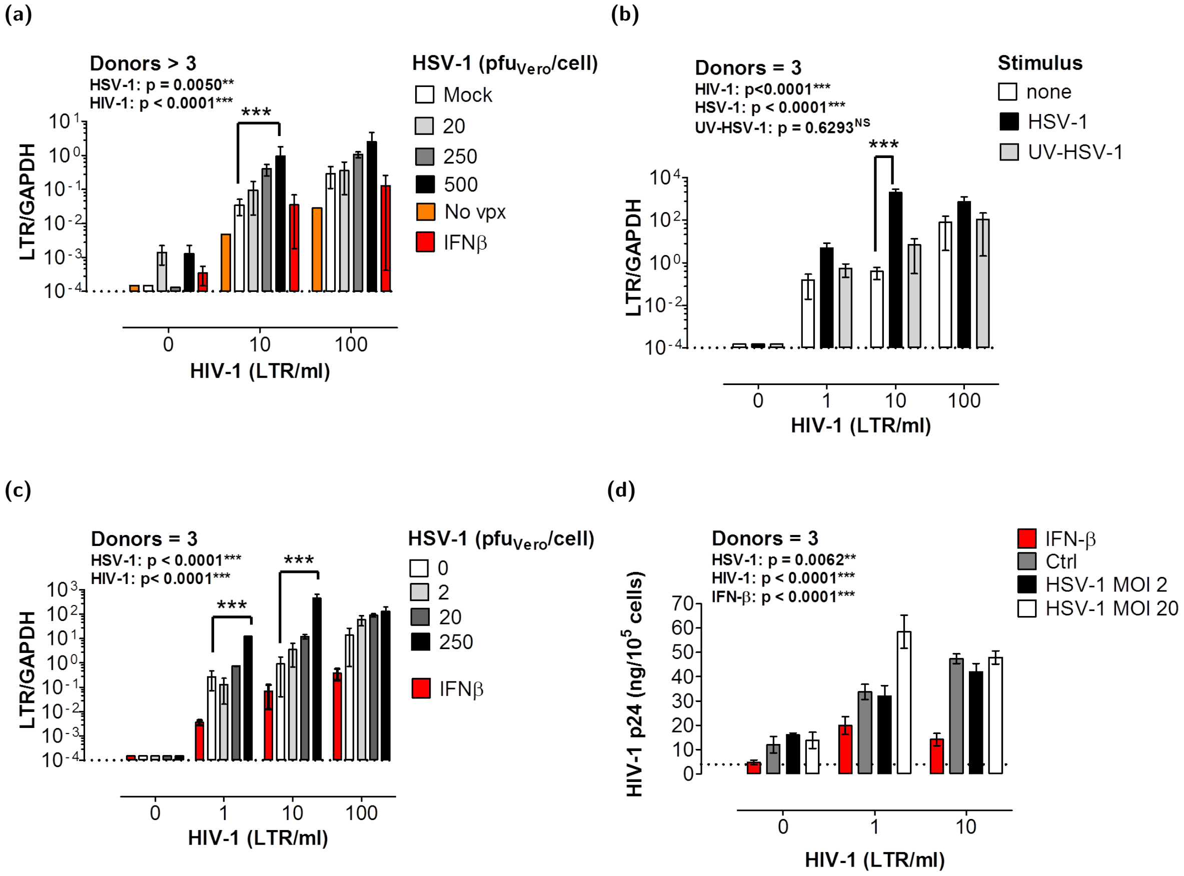

Figure 30: The effect of HSV-1 superinfection on HIV-1 infection.
(a) and (b): Macrophages were infected with single round HIV-1. 6dpi, the macrophages were super-infected with HSV-1 (a) and/or stimulated with UV-HSV-1 (b). 24 hours after infection with HSV-1, total cellular RNA was extracted, DNase treated and reverse-transcribed into cDNA. GAPDH and LTR expression levels were assessed by RT-qPCR. LTR transcript levels were normalised to GAPDH expression. (c): Macrophages were infected with full-length HIV-1. 6dpi, the macrophages were super-infected with HSV-1. 24 hours after infection with HSV-1, total cellular RNA was extracted, DNase treated and reverse-transcribed into cDNA. GAPDH and LTR expression levels were assessed by RT-qPCR. LTR transcript levels were normalised to GAPDH expression. (d): Macrophages were infected with full-length HIV-1. 6 days post infection the macrophages were super-infected with HSV-1 or stimulated with UV-HSV-1. After 24 hours, the supernatents were collected and HIV-1 virion release assessed by p24 ELISA. In all of the graphs, each bar represents the mean of at least 3 donors. Error bars = SEM. 2 way ANOVA p values are shown for the effect of HSV-1, HIV-1 and IFN-β, with the Vpx control excluded if necessary.

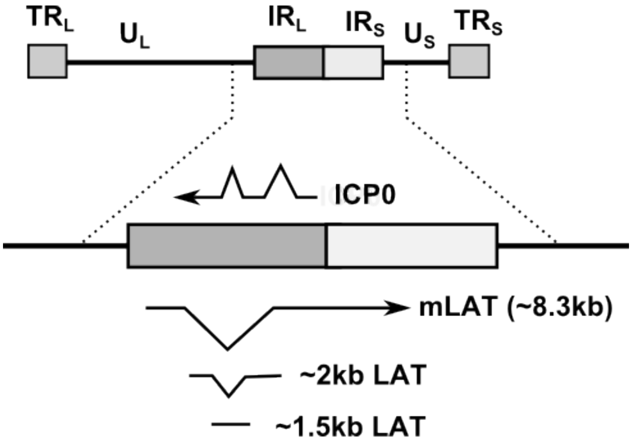

Figure 31: Organisation of the HSV-1 genome
The gene for the latency-associated transcripts (LATs) is diploid and located in the repeat regions flanking the unique long (U$_L$) sequence of the dsDNA genome. The minor (primary) latency associated transcript (mLAT) is transcribed from the opposite strand to ICP0. The major LATs are introns of 1.5 kb and 2 kb that are spliced from mLAT. IR, internal repeat; TR, terminal repeat; U$_L$, unique long sequence; U$_S$, unique short sequence.

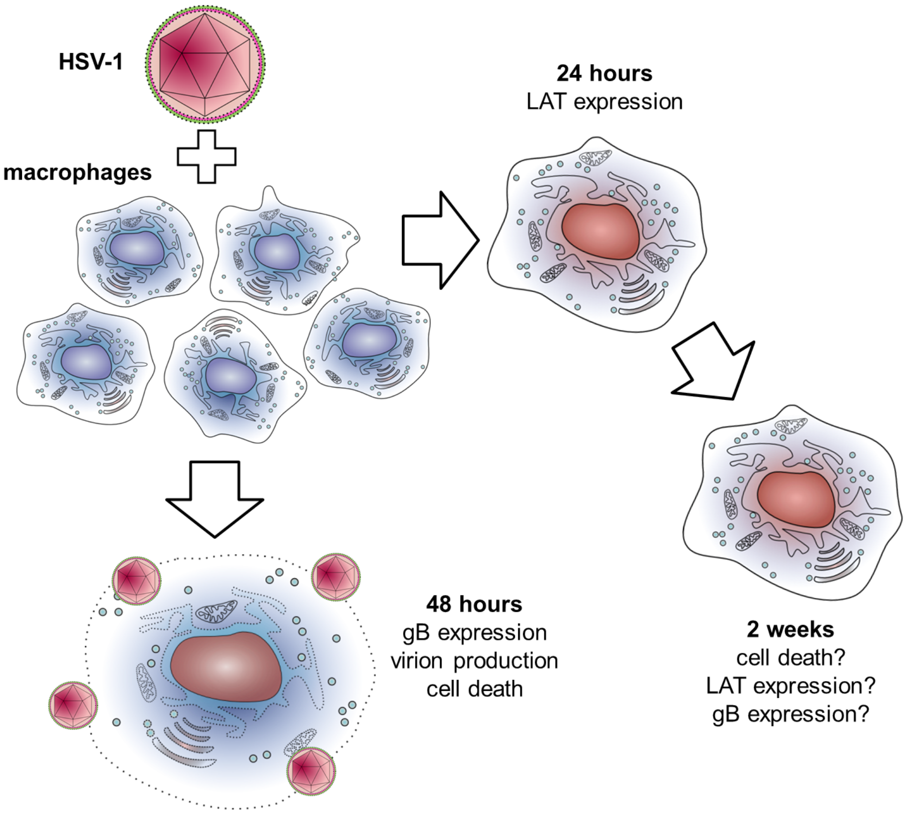

Figure 32: Model of HSV-1 latent infection of macrophages.
Experimental model to investigate whether HSV-1 establishes latency in macrophages. If this is the case we would expect gB expression to decrease over time and LAT expression to persist and stabilise.

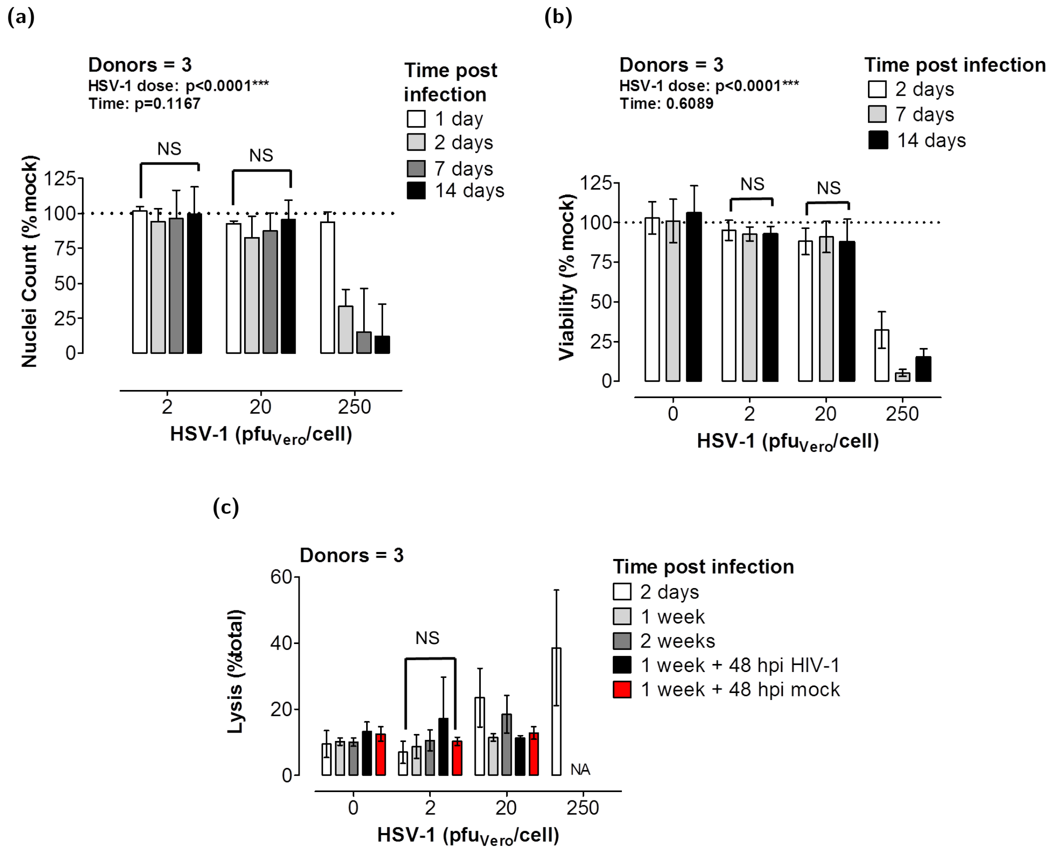

Figure 33: Survival of HSV-1 infected macrophages.
(a): Macrophages were infected with various doses of HSV-1. At various time points post infection the cells were fixed, stained with DAPI and imaged using an automated fluorescent microscope. For each well of the plate, 23 non-overlapping images were taken. The number of nuclei per image was counted using image analysis software and added to give the total number of nuclei per well. The total number of nuclei in an infected well of macrophages is represented as a percentage of the total number of macrophages in a mock infected well. Each bar represent the mean of 3 donors. Error bars = SEM. 2 way ANOVA p values are shown for the effect of HSV-1 dose and time. (b) and (c): Macrophages were infected with HSV-1. 2, 7 and 14dpi, the cell viability was assessed by the alamarBlue assay (b) or the supernatents collected to assess cell lysis by LDH ELISA (c). Each bar represents the mean of 3 donors. Error bars = SEM. 2 way ANOVA p values are shown for the effect of HSV-1 dose and time. NA = not analysed

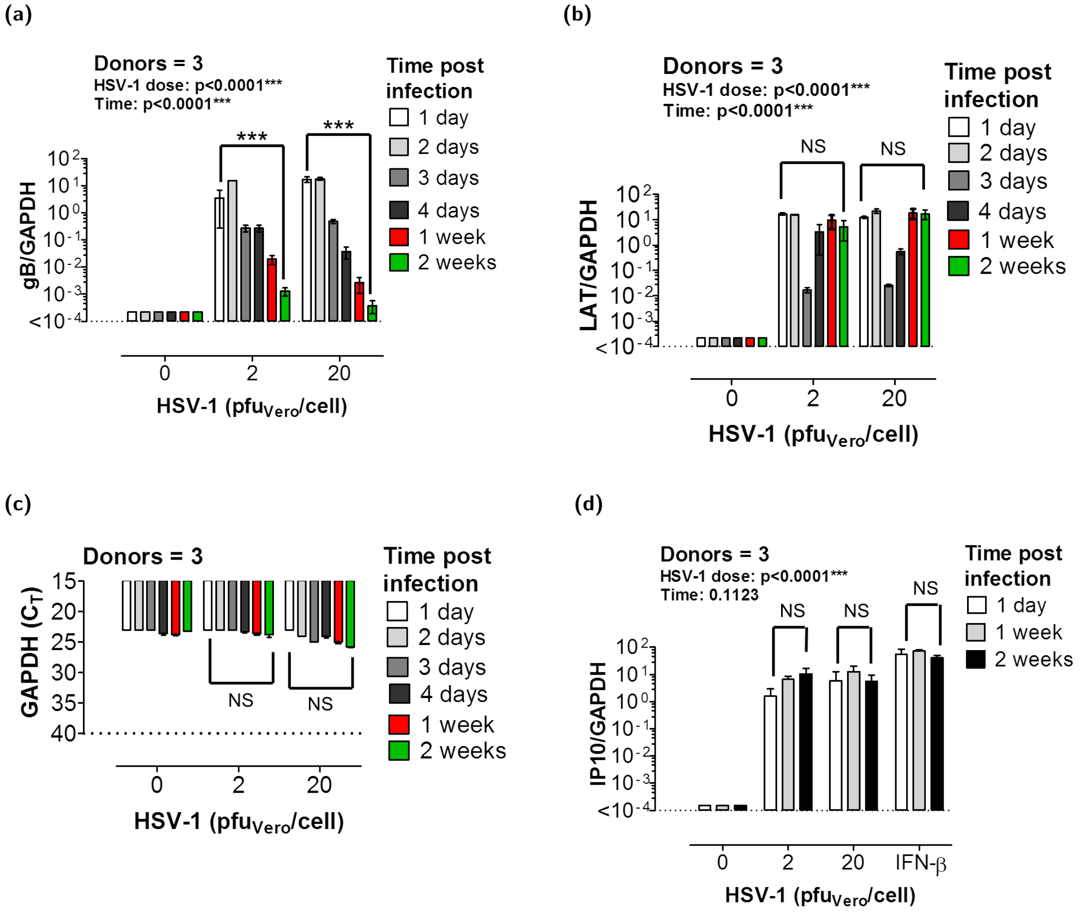

Figure 34: Transcription in HSV-1 infected macrophages over time.
(a) to (d): Macrophages were infected with HSV-1. At various time points post infection, total cell RNA was extracted and GAPDH (c) and HSV-1 gB (a), LAT (b) or IP10 (d) expression determined by RT-qPCR. HSV-1 and IP10 transcript levels were normalised to GAPDH expression. Each bar represents the mean of 3 individual donors. Error bars = SEM. 2 way ANOVA p values are shown for the effect of HSV-1 dose and time.

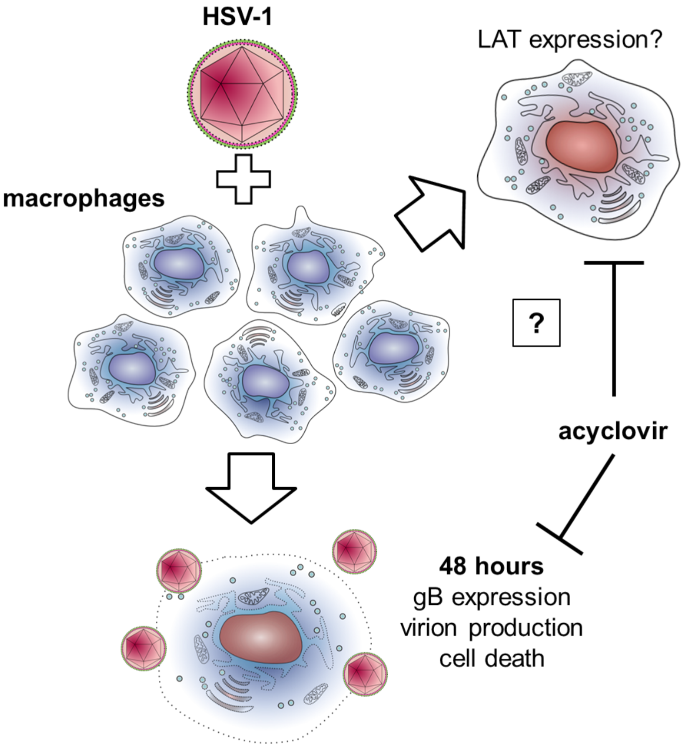

Figure 35: The effect of acyclovir on HSV-1 latency.
An experimental model to assess the effect of acyclovir on HSV-1 latency in macrophages. Macrophages were infected with HSV-1 in the presence or absence of ACV. The effects of the drug on HSV-1 replicative infection and latency were assessed by quantifying gB and LAT expression over time.

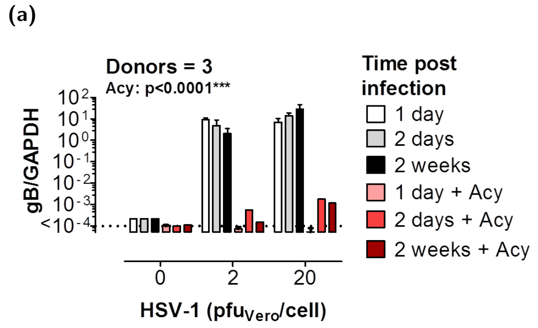

Figure 36: The effect of acyclovir on HSV-1 latency.
Macrophages were infected with HSV-1 in the presence or absence of acyclovir. At various time points post infection, total cellular RNA was extracted, DNase treated and reverse-transcribed into cDNA. GAPDH, HSV-1 gB expression was assessed by RT-qPCR. Expression of viral genes were normalised to GAPDH expression. Each bar represents the mean of 3 individual donors. Error bars = SEM. The 2 way ANOVA p values are shown for the effect of acyclovir on HSV-1 gene expression, at a dose of both 2 and 20pfu$_{vero}$/cell.

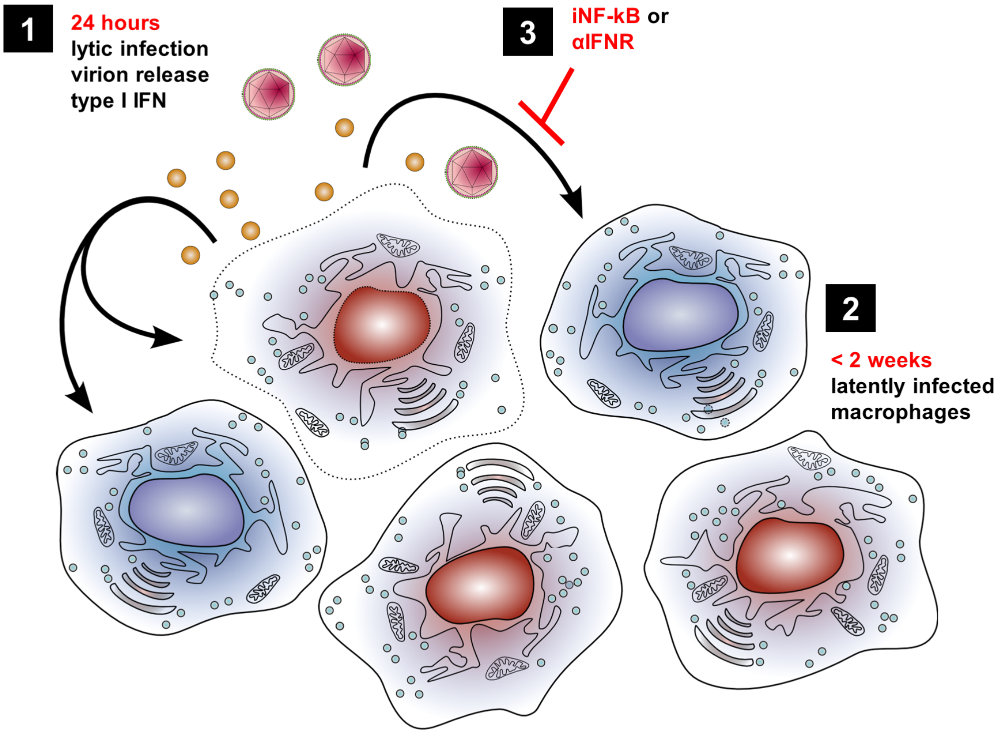

Figure 37: The effect of blocking the IFNR on HSV-1 latency.
Experimental Model. [1] HSV-1 infection of macrophages is replicative, and leads to cell death, virion release and induction of a type I IFN response. [2] IFN-β release by HSV-1 infected macrophages induces a type I IFN response in uninfected macrophages within 24 hours. Virion release from infected macrophages does not occur until 48hpi. Secondary HSV-1 infection therefore occurs in already activated macrophages, potentially restricting HSV-1 infection and leading to the establishment of latency. [3] HSV-1 replication and the HSV-1 dependent response can be inhibited using an NF-κB inhibitor or an IFNR blocking antibody respectively.

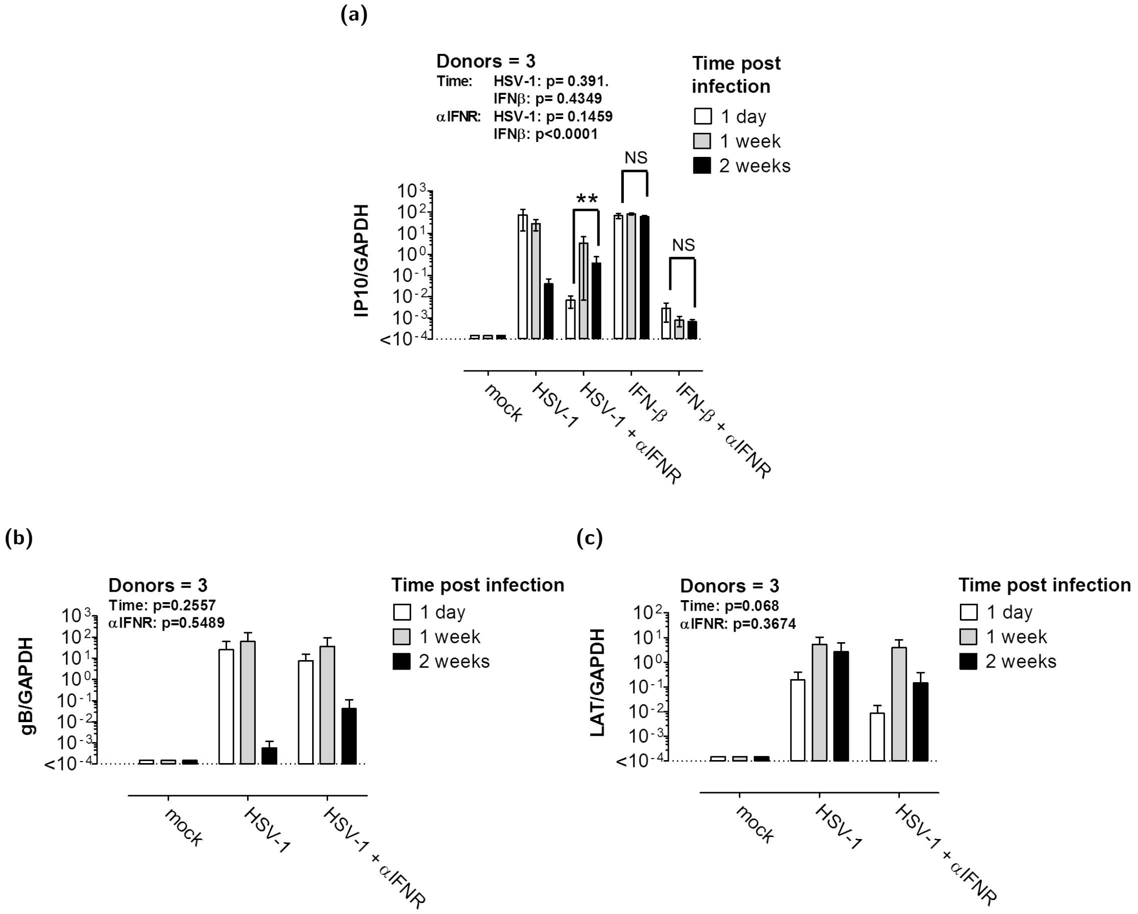

Figure 38: The effect on HSV-1 latency of blocking the type I IFN receptor.
(a) to (c): Macrophages were infected with HSV-1 (2pfu$_{vero}$/cell) in the presence or absence of an IFNR blocking antibody. After 24 hours the antibody was removed. 24 hours or 1 week post infection, total cellular RNA was extracted, DNase treated and reverse-transcribed into cDNA. GAPDH, IP10 (a), HSV-1 gB (b) and LAT (c) expression were assessed by RT-qPCR. Expression of viral genes were normalised to GAPDH expression. Each bar represents the mean of 3 individual donors. Error bars = SEM. The 2 way ANOVA p values are shown for the effects of time and αIFNR.

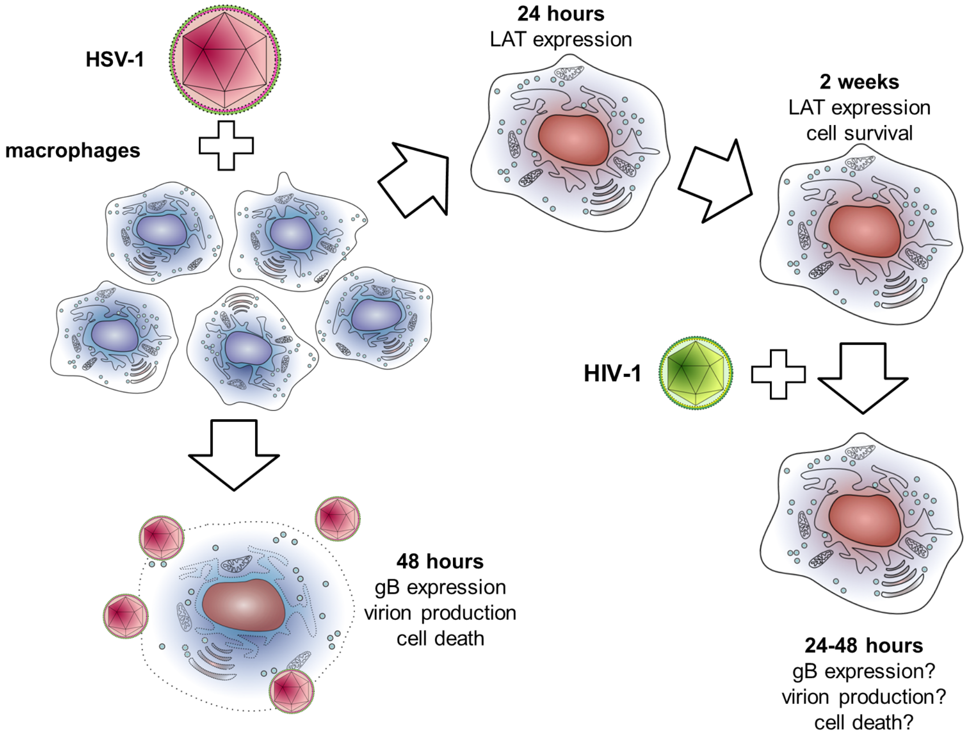

Figure 39: An experimental model to assess whether HIV-1 can reactivate latent HSV-1.
Macrophages were infected with HSV-1. After 2 weeks, the macrophages that remained were exposed to HIV-1 or stimulated with LPS. After 24 hours, HSV-1 reactivation was assessed by measuring HSV-1 gB and DNA pol expression and virion production.

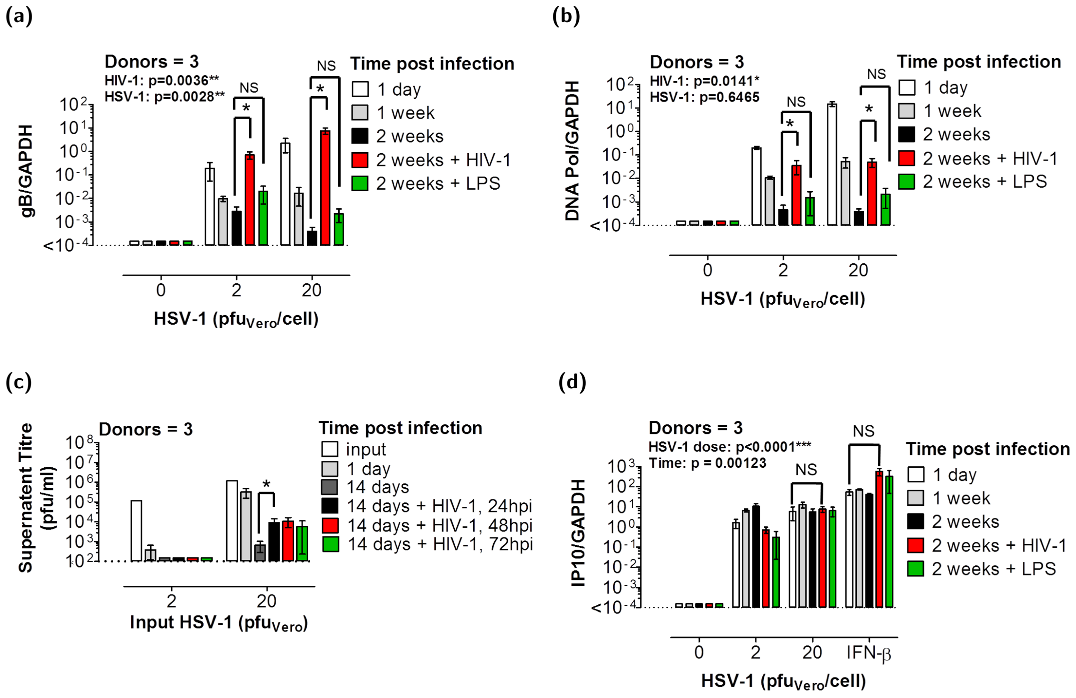

Figure 40: HIV-1 reactivates latent HSV-1.
(a) and (b): Macrophages were infected with HSV-1. After 14 days the infected cells were super-infected with HIV-1 or mock infected. At various time points post infection, total cellular RNA was extracted, DNase treated and reverse-transcribed into cDNA. GAPDH and HSV-1 gB (a) or DNA pol (b) expression were assessed by RT-qPCR. gB and DNA Pol transcript levels were normalised to GAPDH expression. (c): The supernatent was collected from the infected macrophages and assessed by plaque assay on vero cells for the presence of HSV-1 virions. Each bar represents the mean of 3 individual donors. Error bars = SEM. The 2 way ANOVA p values are shown for the effect of HSV-1 dose or HIV-1 on HSV-1 gene expression levels at 2 weeks post HSV-1 infection, or the effect of time.

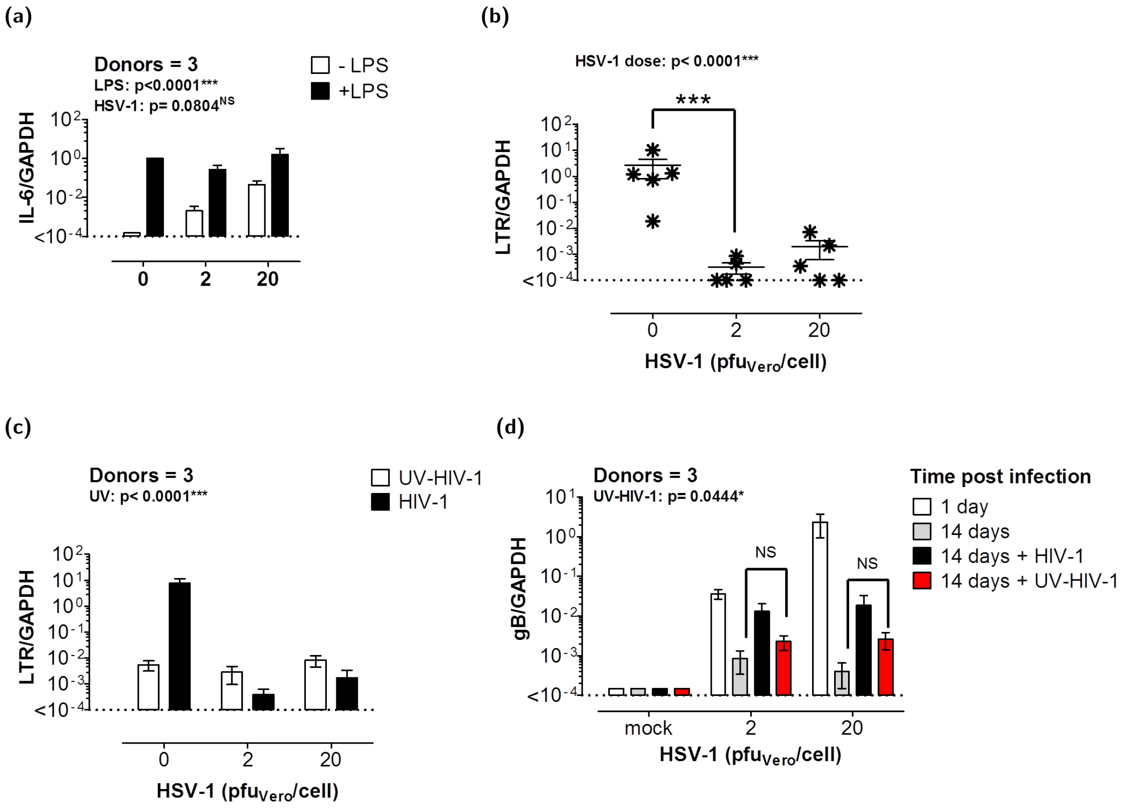

Figure 41: HIV-1 replication is required for HIV-1 dependent reactivation of latent HSV-1.
(a) to (d): Macrophages were infected with HSV-1. After 14 days the infected cells were exposed to HIV-1 or UV-HIV-1, LPS or mock infected. At various time points post infection, total cellular RNA was extracted, DNase treated and reverse-transcribed into cDNA. GAPDH and IL-6 (a), HIV-1 LTR (b) and (c) and HSV-1 gB (d) expression were assessed by RT-qPCR. gB, LTR and IL-6 transcript levels were normalised to GAPDH expression. Each bar represents the mean of 3 individual donors. Error bars = SEM. The 2 way ANOVA p values are shown for the effect of UV-HIV-1 on HSV-1 gene expression, or LPS and HSV-1 on IL-6 gene expression.

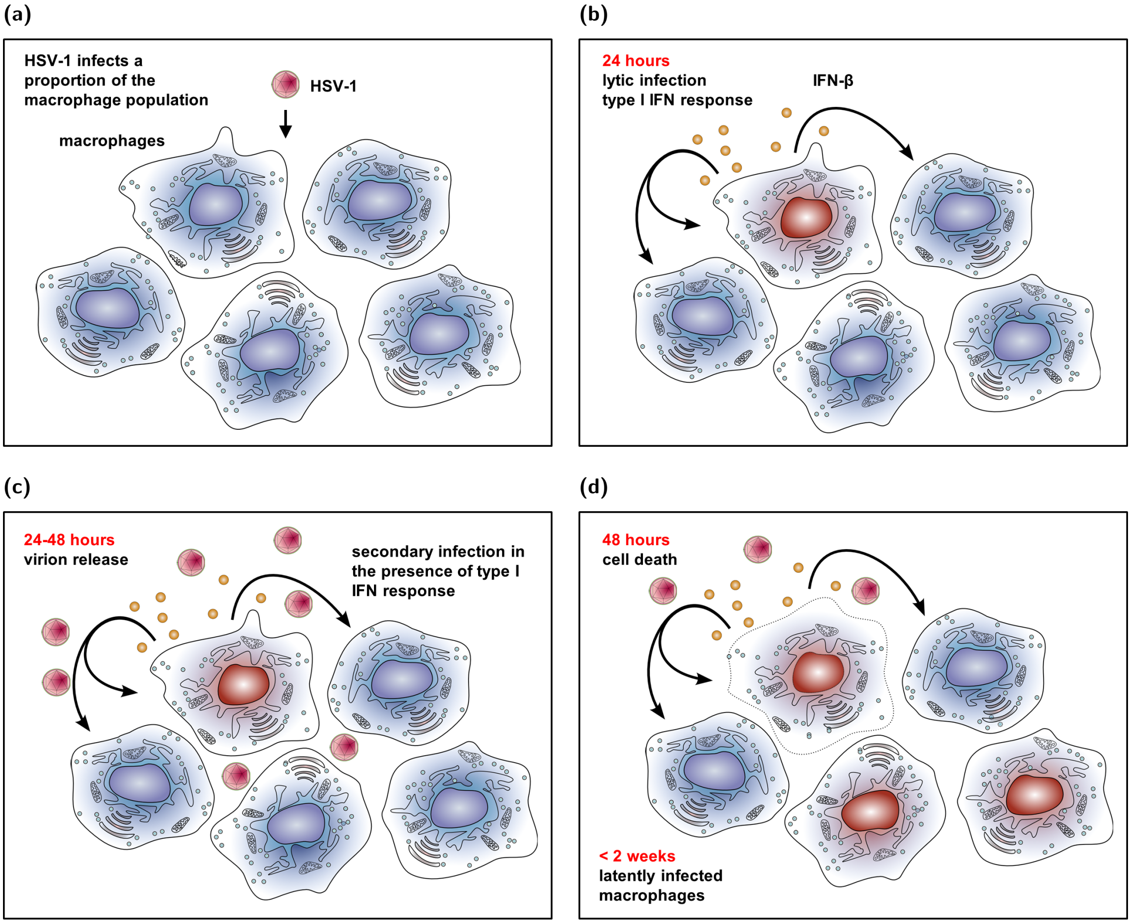

Figure 42: A model for the establishment of latency in macrophages.

Table 1: Cytoplasmic DNA sensors.

Name(s)	**Ligand/pathogen(s)**	**Cell types**	**Response**	**Adapter(s)/signal molecules**
ZBP-1/DAI/DLM(552)	Z-DNA(553–555), B-DNA(47), dsDNA, poly(dA:dT)(555), long dsDNA(555) HCMV(556), HSV-1(556)	L929 fibroblast and HEK293 cells(557,558)	Type I IFN response(555)	IRF3, TBK1(47)
AIM2(48,559–562)	dsDNA AdV, HSV-1, Francisella tularensis(563,564), Porphyromonas gingivalis(565), Mycobacterium bovis(566), vaccina virus(567), Listeria monocytogenes(567–569), MCMV(567), Mycobacterium tuberculosis(570)	THP-1(560,565), murine macrophages(566), human macrophages(563) and cancer cells(571)	Pyroptosis(560)	ASC(560)
Pol III(572)	poly(dA:dT)(572) EBV(572), not HSV-1(191)	HEK293T, human MoDC, primary monocyte, MEF and L929 fibroblast cells(572)	Produces an RNA intermediate that is detected by cytoplasmic RNA sensors and induces a type I IFN response	MAVS, RIG-I(572)
Extrachromosomal H2B(557)	poly(dA:dT), poly(dG:dC)(557) HPV (types 16 and 18) and AdV5(557)	HEK293T cells(557)	Type I IFN(557)	CIAO, MAVS(557)
IFI16/p204(49)	dsDNA, DNA damage(573,574) Vaccina virus(49), HIV-1(575,576), HSV-1(577), HSV-2(578), HCMV(56) (579), KSHV(580)	THP-1(49), CD4$^+$ T(575), fibroblast(573) and HFF cells(577)	Type I IFN(49) and cell death(575)	STING, IRF3, NF-κB(49)
HMGBs(581)	All nucleic acids, including poly(dA:dT)(581), CpG oligo-deoxynucleotide)(582,583), base-free phosphorothioate deoxyribose homopolymer(584), poly(I:C) ssRNA poly(U), ssRNA-5'triphosphate(581) HSV-1	MEF, RAW264.7 macrophage and NIH/3T3 fibroblast cells(581)	Type I IFN and inflammatory cytokine production(581)	co-reptors/co-ligands for other nucleic acid receptors(581)
LRRFIP1(585)	dsRNA(586), poly(dC:dG)(587), poly(dA:dT)(585) Listeria monocytogenes, VSV(585)	monocyte(588) and macrophage(585) cells	Type I IFN response(585)	β-catenin(585,589)
cGAS	dsDNA(50,56) HIV-1, HIV-2(590,591), AdV(592), MLV(591), SIV(591), HSV-1(56)	DC(590), murine MS1 endothelial, RAW 264.7 macrophages(592) and THP-1(591) cells	Produces the messenger cGAMP to induce a type I IFN response via STING activation	STING

Table 2: Interferons

Group	**Protein**	**Genes**	**Receptor**	**Cell Source**
Type I	IFNα	*ifna1, ifna2, ifna4, ifna5, ifna6, ifna7, ifn8, ifna10, ifna13, ifna14, ifna16, ifna17, ifna21*	IFNAR1/IFNAR2	B cells, T cells, NK cells, macrophages, endothelial cells, pDC, MDDC, epithelial cells, some neurons
	IFNβ	*ifnb1*		
	IFNκ ϵ	*ifnk*		
	IFNω	*ifnw1*		
Type II	IFNγ	*ifng*	IFNGR1/IFNGR2	T cells, NK cells
Type III	IFNλ	*il28a*	IFNLR1	macrophages, pDC, monocyte derived dendritic cells (MDDC), epithelial cells
	IFNλ2	*il28b*		
	IFNλ3	*il29*		

Table 3: Cell death pathways

Pathway	**Description**	**Morphology and characteristics**	**Stimulus,** *receptor(s)*	**Cells**
Necrosis	Uncontrolled cell death, involving energetic catastrophe, lipid peroxidation and osmotic swelling(593)	Cellular swelling, membrane blebs, DNA damage	Cellular stress e.g. heat, cold, UV	All cell types
Pyroptosis	Inflammasome activation and caspase-1 dependent cell lysis(138)	Permeable plasma membrane(594,595), pyknosis, chromatin margination, membrane blebs (plasma membrane detaching from the cytoskeleton due to celllular swelling), no nuclear break-up(139)	HIV-1(429), DNEV(596). *NLPRP3*: K$^+$ efflux, ROS, phagolysosomal destabilization. *NLRP1*: Toxoplasma gondii(597,598), ATP(599), anthrax lethal toxin(600). *NLRC4*: cytosolic bacterial flagelli(601), type III secretion system (T3SS) components(601,602). *AIM2*: dsDNA(48) (560–562)	Many cell types, including: macrophages, DC(595,603), keratinocytes(604), CD4$^+$ T cells(429), monocytes(596) and hepatocytes(50)
Apoptosis(142)	Caspase dependent breakdown of cellular components into apoptotic bodes	Cell shrinkage, membrane blebbing (apoptotic bodies), pyknosis, chromatin margination, karyorrhexis	Extrinsic and intrinsic death receptors (DR), e.g. TNFα receptor TNFα, stauosporine, virus infection	Many cell types
Necroptosis(605)	Programmed cell lysis that operates as an alternative host defence strategy to both apoptosis and pyroptosis(145) (128)	Indistinguishable from necrosis	Inhibition of caspase-8(148,149), TRAIL (*TRAILR*)(606), CD95L (*CD95*), TNFα (*TNFR*)(607), IFNγ (*IFNγR*), CD3 (*TCR*)(608), CD28 (*TCR*), LPS (*TLR4*)(412), dsRNA (*TLR3*)(609), genotoxic stress, type I IFN(408), anticancer drugs(610), dsDNA (*ZBP-1*)(409), poly(I:C) (*RIG-I*)(410–412)	Many cells, including macrophages(597), astrocytes(611), hepatocytes(612), epithelial cells(613) and multiple organs, including the heart(614), the liver(612), the brain and the kidney(615)

Table 4: PRRs that detect HSV-1

HSV PAMP	PRR	Cell	Response	References
gB	TLR2	HEK 293T	Inflammatory cytokine	(428)
gH/gL	$\alpha v\beta 3$ integrin/TLR2	neurons, keratinocytes	Type I IFN	(426)
gD	unknown	human PBMC, human MDDC	Type I IFN	(616,616)
virion component	mannose receptor	human MDDC	Type I IFN	(617)
cytoplasmic dsRNA	RLRs	murine macrophages, MEFs	Type I IFN	(618)
	Mda5	human macropahges	Type I IFN	(191)
endosomal dsRNA	TLR3	fibroblasts	Type I IFN	(207)
endosomal DNA	TLR9	mouse pDC	Type I IFN	(619,620)
	TLR9	human pDC	Type I IFN	(621)
cytoplasmic DNA	RNA Pol II/RIG-I	murine macrophages	Type I IFN	(622)
	IFI16	murine macrophages	Type I IFN	(49)
	DAI	murine macrophages, L292 cells	Type I IFN	(47)
	DHX36	human pDC cell line	Type I IFN	(623)
	cGAS	THP1s	Type I IFN	(143)

Table 5: HSV dependent cell death

Pathways	**Mechanism(s)**	**Evidence**	**Contradictory data**
Caspase dependent apoptosis	ICP27 activation of apoptosis via p38 dependent Caspase-3 activation(399).	Stable expression of ICP27 in HeLa cells causes a cell growth defect associated with apoptosis(399).	ICP27 and U(S)3 have both been identified as anti-apoptotic factors(400).
	U(S)3 dependent inhibition of the pro-apoptotic factor PDCD4	In HeLa and HEp-2 cells, U(S)3 post-translationally modifies PDCD4, causing it to accumulate in the nucleus.	
	ICP10 dependent induction of apoptosis.(624)	Expression of ICP10 induces apoptosis in Jurkat cells(624).	
Caspase independent apoptosis(625,626)	HSV-1 dependent mitochondrial cyt c release.	In HSV-1 infected HEp-2 cells, cyt-c release is observed before caspase-9 and caspase-3(625) and pan-caspase inhibitors do not prevent HSV-1 dependent cyt-c release.	A decrease in PARG levels can enhance auto-PARylation mediated inhibition of PARP and thereby act as an anti-apoptotic mechanism. In this way ICP0 may prevent cell death via virus-mediated PARylation early in the HSV replication cycle(626).
	HSV-1 dependent manipulation of PARP and PARG protein activity(626).	HSV-1 infection decreases cellular levels of NAD$^+$, and thereby increase PARylation by PARP family members.	
	Degradation of PARG, the cellular protien that removes PAR chains proteins targeted by PARP, PAR chain accumulation induces AIF dependent, caspase-independent apoptosis.	ICP0 degrades PARG.	
Pyroptosis(223)	Recognition of HSV-1 DNA by AIM2	HSV mutant ΔPK has strong ontolytic activity against pyroptotic caspase-1 activation(223).	IL-1β is not released following HSV infection(406).
Autophagy dependent cell death.	Dependent on recognition of viral DNA in the cytoplasm by stimulator of interferon genes (STING)(627)	HSV-1 activates autophagy in non-permissive cells murine myeloid cells, including BMDDCs and RAW 264.7 macrophages(627), this has been linked to HSV-dependent cytolysis(223).	
Necroptosis	ICP4 or U(S)3 dependent(224).	In U937 cells infected with a HSV ICP4 and U(S)3 mutant strain d120, the cell death observed could be reduced by necroptotic inhibitors(224).	

Table 6: Factors involved in HSV-1 dependent cell survival and inhibition of apoptosis

Anti-apoptotic factor	**Function in HSV replication**	**Anti-apoptotic effect(s)**	**Mechanism(s) of action**
ICP27	Regulatory function in virus replication after induction of E gene expression and viral DNA synthesis(628).	Infection of HeLa cells or HEp-2 cells with a HSV-1 ICP27 deletion mutant induces apoptosis, whereas wild-type HSV-1 infection does not(400).	Activation of JNK signalling(399,629,630).
U(S)3(631)	An HSV protein with structual homology to the cellular protein PKA. Important for viral egress(632).	(1) HSV-1 U(S)3 deletion mutants cause apoptosis in murine neurons(414). (2) Blocks cell death and caspase activation induced by over-expression of Bax, Bad and Bid(415,416).	(1) Post-translational modification of Bad(415) or inhibition of downstream apoptotic signalling(416). (2) Caspase-8, p53 dependent and phospho-JNK independent(414).
ICP10PK	An HSV-2 serine/theroine kinase and homologue of small heat shock (sHS) H11/HspB8(633). Required for productive infection and reactivation from latency.(634–637)	(1) Protects neurons from extrinisic death signals or absence of survival signals(638,639). (2) In neurons, prevents paracrine apoptotic signalling(640). (3) In HeLa and HEp-2 cells, blocks TNF-α, FasL, cycloheximide and poly(I:C) Caspase-8 mediated apoptosis(624,641,641).	(1) Activation of survival signalling pathways e.g. the Ras/MEK/ERK pathway(642–645). (2) Blocks AIF release(640). (3) Unknown, but independent of survival signaling promotion(624,641).
gD	Component of the virion envelope and essential for cell entry of HSV-1 virions.	In human neuroblastoma cells, inhibits HSV-1 dependent cell death(646,647) and protects U937 cells from FasL mediated apoptosis(648).	gB ligation of HVEM activates NF-κB signaling, inhibits caspase-8 activation and prevents up-regulation of pro-survival factors(455,648)
LAT	Has a role in maintenance of latency and re-activation	(1) Protects cells from serum starvation. (2) Promotes cell survival. (3) Block apoptosis from extrinsic signals provided by cytotoxic CD8$^+$ T cells(649)	(1) Inhibits Caspase-3 activation. (2) Increases protein kinase B (PKB/Akt) levels(650). (3) Blocks GrB/perforin-induced activation of caspase-3(649).
NF-κB	Activation is essential for efficient HSV gene expression(651).	Activation blocks extrinsic pro-apoptotic signals in HSV-1 infected cells(648,652–654) and reduces HSV-1 mediated cell death *in vivo*(655).	Translocates to the nucleus following HSV-1 infection, with a timing that coincides with E gene expression and apoptosis prevention(652)
PI3K/Akt	Stimulates HSV IE protein expression(656) (657).	In human oral epithelial cells, inhibition of PI3K phosphorylation increases HSV-1 dependent DNA fragmentation, PARP cleavage and Caspase 3/7 activation(657).	PI3K/Akt is activated by the HSV tegument protein VP11/12(658), but it is unknown whether this underlies the antiapoptotic effect.

Table 7: Viral factors involved in HIV-1 transcription

Viral factor	**Mechanism**	**References**
LTR	A DNA sequence located at the 5' end of the HIV-1 provirus. Contains a canonical TATA box sequence followed by 2 NF-κB and 3 Sp1 binding sites. These constitute the core HIV-1 promoter to which and cellular RNAPII, transcription factors and transactivators are recruited.	(659–662)
TAR	A 59 nucleotide stem-budge loop structured RNA element located at the 5' end of all HIV-1 transcripts. Functions as a transactivation responsive region. Recruits tat and associated cellular factors to promote completion of transcription initiated at the LTR promoter.	(663)
Tat	An HIV-1 accessory protein that plays a highly specific role in the recruitment of cellular transcription factors and enables elongation of HIV-1 transcript by recruiting cellular cofactors, including the P-TEFb complex, TFIID and TCERG1, to TAR.	(664–670)
Nef	A HIV-1 accessory protein that indirectly increases LTR transcription by positively regulating cellular transcription factors including NF-κB, NF-AT and AP-1. Also up-regulates expression a cellular heat shock protein, HSP40, that binds to the LTR promoter and enhances LTR transcription.	(539,671)
Vpr	An HIV-1 late gene product that mediates enhanced viral expression by indirectly arresting the cell cylce in the G2/M phase, and directly via transactivation of the LTR.	(672–674)

Table 8: Cellular factors involved in HIV-1 transcription

Cellular factor	**References**	**Additional information**
P-TEF-b	(675)	A key regulator of the process controlling the processivity of RNA polymerase II(676) that is recruited to the HIV-1 LTR by tat.
CDK9	(675)	A component of the P-TEFb that promotes elongation of the LTR transcript by phosphorylating the Ser2 residue within the CTD of cellular RNAPII, leading to the release of arrested RNA complexes in the 5' regaion of the LTR.
TCERG1	(677,678)	A nuclear protein that promotes elongation of the LTR transcript by modulating cellular RNAPII activity.
Sp1	(679–682)	A zinc finger transcription factor that binds to GC-rich motifs of many promoters. Involved in many cellular processes, including cell differentiation, apoptosis, immune responses and chromatin remodeling and is critical for *in vivo* transcriptional regulation of HIV.
IRF-1/2	(679,680)	Member of the interferon regulatory transcription factor (IRF) family, activates type I IFN and ISG transcription(683).
CREB-1/p300	(684–687)	Member of the leucine zipper family of DNA binding proteins. Binds the cAMP-responsive element and induces transcription of genes in response to stimulation of the cAMP pathway, for example by IL-6 or ICAM-1.
AP-1	(684,688)	Tat enables co-operation of NF-AT with this transcription factor.
NF-κB	(689,690)	A cellular transcription factor activated by a broad array of signalling events, including type I IFN and cytokine receptor signalling.
NF-AT	(679,680,691)	A family of transcription regulators that plays a necessary role in T cell activation via control of IL-2 gene activation. Bind specific sequences in the LTR promoter and synergizes with NF-κB and tat to activate HIV-1 transcription.
HSF-1	(671)	A cellular heat shock protein that enhances LTR transcription directly, and indirectly by enhancing Nef mediated upregulation of HSP40.

Table 9: Lentivector plasmids for transfection

Lentivirus	**Plasmids**
SIV3+ (Vpx)	pMDG (VSV-G), SIV3^{+}
ΔEnv R9 BaL	pMDG (VSV-G), ΔEnv R9 BaL, p8.9
R9 BaL	R9 BaL

Table 10: Function of glycoprotein B

Function	**Details/mechanism**	**References**
Evasion of the immune system	HSV-1 infection of APC rapidly decreases CD1d surface expression and inhibits recognition by CD1d restricted NKT cells. In CD1d expressing HeLa cells gB interacts with CD1d in the ER and stably associates with it during intracellular trafficking through the TGN, preventing the recycling of CD1d back to the cell surface after endocytosis.	(388)
	In a human melanoma cell line, gB binds MHC class II molecule HLA-DR and diverts it into the endosomal pathway.	(387)
A fusion protein during virion entry	gB has structural homology to other viral fusion proteins, fusion potential and along with gD, gH and gL, is one of the HSV-1 glycoproteins essential for virion entry.	(382–385)
Endosomal entry	An acidic pH triggers conformational change and formation of the oligometric state of gB pre-fusion forms. This is separable from its fusion activity and is linked to gB's ability to facilitate entry of the virion into the cytosol from the low pH endosome.	(692–694)
Facilitates entry by binding specific cell surface molecules	PIRRα and MAG both function as gB receptors. gB O-glycans at residues Thr53 and Thr480 associate with PIRRα. Mutation of these residues decreases HSV-1 replication in the cornea of corneal inoculated mice and decreases herpes stromal keratinitis and neuro-invasiveness - suggesting that gB plays an important role in facilitating HSV-1 spread and infectivity independent of other viral glycoproteins.	(695,696)
Endosomal location of gB is with disease	The Thr-887 residue in the cytoplasmic tail of gB can be phosphorylated by the HSV-1 protein U$_S$3 kinase, leading to decreased surface expression and increased endosomal accumulation of gB. This is associated with stromal keratitis and neuro-virulence in mouse models of HSV-1 infection.	(697,698)
Cell-to-cell fusion	gB expression on the cell surface facilitates cell fusion and the formation of giant syncytia during HSV-1 infection. Trafficking of gB to the TGN reduces cell fusion.	(699–701)

Table 11: Functions of ICP0

Function	**Details/mechanism**	**References**
Activation of viral gene expression	ICP0 transactivates IE, E and late gene expression through its E3 ligase function, dispersal of the HDAC1/CoREST/REST/LSD1 repressor complex and resultant acetylatation of histones at virus promoters. ICP0 also recruits cyclin D3 to the sites of viral replication in the nucleus.	(393,702–706)
Reactivation of latency	ICP0 is necessary, and sufficient in combination with ICP4 and VP16, for the efficient reactivation of HSV-1 in primary cultures of latently infected TG cells. The ICP0 promoter can be activated by stress induced cellular transcription factors.	(390–392,467,468)
Evasion of intrinisic cell defences	Following entry of the viral genome into the nucleus, ND10 and DNA damage proteins are recruited by the cellular ubiquitin ligases RNF8 and RNF168, silencing the genome. ICP0 targets RNF8 and RNF168 for degradation, enabling IE gene expression. ICP0 also degrades the ND10 components PML and SP100.	(705,707–710)
Evasion of inflammatory cytokine response	Inhibits NF-κB downstrean of TLR ligation, by mediating translocation of USP7 from the nucleus to the cytoplasm. USP7 binds and deubiquitinates TRAF6 and IKKγ and thereby termiates NF-κB signalling	(425)
	Inhibits TNF-α mediated NF-κB activation by facilitating degradation of p65 and preventing p50 nuclear translocation upon TNF-α stimulation. This function of ICP0 requires the ring finger domain.	(424)
	Reduces the inflammatory cytokine response to TLR2 ligation by decreasing the levels of MyD88 and TRIAP.	(711)
Evasion of the type I IFN response	During *in vivo* infection, ICP0 is necessary to prevent Stat-1 dependent type I IFN responses in HSV-1 infected mice. ICP0$^-$ HSV-1 is hypersensitive to type I IFN treatment in tissue culture	(712–714)
	ICP0 has been shown in combination with vhs, to inhibit IRF3 and IRF7 mediated induction of ISG transcription, via both Ring Finger domain dependent and independent mechanisms.	(715–718)
	Some observations indicate that ICP0 degrades the cytoplasmic DNA sensor IFI16 and prevents nuclear IFI16 induction of IRF3 signalling. However, these results remain controversial.	(719)
Evasion of adaptive immunity	Expression is sufficient to degrade CD38 in mature dendritic, reducing the ability of these cells to stimulate T cells.	(720–722)
Dismantling of the the microtubule network	In a mechanism dependent upon the ring finger domain, ICP0 dismantles the microtublule network of the host cell late on during HSV-1 infection, preparing the cell for virion synthesis and egress.	(723)
NK mediated cell lysis	ICP0 expression is sufficient to increase the susceptibility of HSV-1 infected cells to NK cell mediated lysis by upregulating cell surface expression of the NCR ligands NKp30, NKp44, and NKp46.	(724)
Virion entry	ICP0 is contained in the tegument of HSV-1 capsids and is necessary for the targeting of incoming capsids to the nucleus.	(725)

Table 12: LTR transcription activation by co-infecting pathogens and drugs

	Drug/pathogen	**Mechanism**	**Ref.**
Herpesviruses	KSHV	ORF50, an IE protein important for KSHV reactivation from latency(437), interacts synergically with tat to enhance LTR transcription.	(438,439)
	HCMV	CMV IE1/2 genes activate LTR transcription by an unknown mechanism.	(445,726,727)
	VZV	The IE4 protein trans-activates LTR transscription via a NF-κB dependent mechanism.	(441)
	EBV	The EBNA2 protein, important for the establishment and maintenance of EBV latency in B cells(442), enhances LTR transcription vai a Sp1 and NF-κB dependent mechanism.	(443)
	HHV-6	HHV-6 co-infection of HIV-1 infected CD4$^+$ T cells or human fetal astrocytes enhances HIV-1 LTR transcription, via an NF-κB dependent mechanism.	(444,445)
Other viruses	HBV	HBX activates HIV-1 transcription by stimulating binding of C/EBPβ and CREB1/2 to cis-regulatory elements in the HIV-1 LTR, such that NF-κB and NF-AT are recruited.	(728)
	AdV	13S E1A, activates LTR transcription via a p300 independent TBP dependent mechanism.	(729)
	HCV	Co-infection of wild-type virus with HIV-1 in hepatocytes enhances LTR transcription and HIV-1 gene expression by an unknown mechanism, although may involve interaction of the HCV protein NS3 with HIV-1 vpu.	(730,731)
Bacteria	*Mycobacterium tuberculosis*	Rv1168C, a proline-proline-glutamic acid protein with unknown function(732) that enhances LTR transcription by ligating TLR2 and activating NF-κB signalling.	(446–448)
	Intracellular bacteria	*Salmonella enteritidis, Yersinia enterocolitica, Legionella pneumophila* and *Escherichia coli* all increase HIV-1 transcription in macrophages, although only *Salmonella enteritidis* also increases HIV-1 production. LPS stimulates HIV-1 transcription in macrophages but not T cells.	(733)
	Neisseria gonorrhoeae	Increases HIV-1 transcription in Jurkat CD4 T cell and DC, via TLR2 ligation and NF-κB dependent mechanism.	(449–451)
Fungi	*Cryptococcus neoformans* and *Candida albicans*	Increases HIV-1 transcription in human monocytes, via an NF-κB dependent mechanism.	(452)
Protocists	*Plasmodium falciparum*	Malarial antigens increase HIV-1 replication in T cells by activating LTR transcription.	(734)
	Toxoplasma gondii	Increases HIV-1 transcription in murine macrophages *in vivo*, via an NF-κB dependent mechanism.	(453)
	Leishmania donovani	The lipophosphoglycan surface molecule increases HIV-1 transcription in myeloid cell lines, via a mechanism involving TNF-α.	(735)
Drugs	Histone deacetylases inhibitors	Drugs that cause global hyperacetylation and chromatin remodelling, for example rapoxin (TPX) and trichostatin A (TSA) activate HIV-1 transcription in a NF-κB independent manner, involving the disruption of nuc-1 binding of the HIV-1 promoter.	(257–259)
	Retinoic acid	Stimulates HIV-1 transcription in human neuronal SH-SY5Y cells in cooperation with tat.	(736)
	Methamphetamines	Neurotoxins and psychostimulants that enhances LTR transcription by activating NF-κB signalling.	(454)
	1,25-Vitamin D	Ligation of the VDR activates LTR transcription in HeLa, U937, and Cos-1 cells, in an Sp1 and NF-κB independent mechanism.	(737)

Table 13: Transcription factors involved in both HSV-1 and HIV-1 transcription

Transcription factor	**Interaction with HSV-1**
P-TEF-b	Regulates transcription of IE gene promoters. P-TEF-b dependent transcription is promoted by VP16 and inhibited by ICP22(738) and by HSRG1 interaction with Cyclin T2(739).
Sp1	Required for IE and E transcription, phosphorylated and inactivated after TK transcription(519).
CDK9	Required for HSV-1 transcription(740) and is regulated by ICP22(741).
CBP-1/p300	ICP0 recruits CBP/p300 to nuclear structures away from the host chromatin(211). CREB mediated transcription may be involved in reactivation from latency by binding the LAT promoter(742).
CBP-1/p300	ICP0 recruits CBP/p300 to nuclear structures away from the host chromatin(211). CREB mediated transcription may be involved in reactivation from latency by binding the LAT promoter(742).
AP-1	ICP0 increases AP-1 transcription(743). AP-1 may be involved in reactivation from latency by binding the LAT promoter(742).
NF-AT	Activity blocked by HSV-1 infection(744).
NF-κB	VP16 inhibits NF-κB activation to prevent induction of a type I IFN response(426–428). ICP0 inhibits NF-κB activation in response to TNF-α(424).

Table 14: Models and reactivation of HSV-1 latency

Model of latency	**Reactivation**
In vivo animal models of infection	Latently infected ganglion are removed from the animal and cultivated *in vitro* (221,419,503,745–749) UV irradiation eye inoculated with HSV-1 (750) Transient hyperthermia or fever (751–753) Iontophoresis of 6-hydroxydopamine (6-HD), followed by topical instillation of 2% epinephrine to eye incoculated with HSV-1 (754) Sodium butyrate, a histone deacetylase inhibitor Cell trauma (755) Drug induced immune suppression (747,756–759) Anti-apoptotic drugs, e.g. dexamethasone (221) 'Social stress' (760)
In vitro neuronal model: infection of PC12 cells in the presence of ACV and/or NGF	Histone dacetylase inhibitors, e.g Trichostatin A (256,486,487,490,503) Spontaneous or following withdrawl of ACV (256,486,487,503) Removal of NGF (490) Heat-stress (492) Expression of ICP0, VP16, and ICP4 from adenovirus vectors (487) Stimulation with forskolin, an activator of cAMP signalling (487)
HSV-2 quiescent infection of human embryonic lung cells *in vitro*	HCMV super-infection and E gene expression (464)
Human keratinocytes layer containing a model of quiescent neuron infection (PC12 cells)	UV irradiation (504)
Infection of primary superior cervical ganglion (SCG) sympathetic neurons in the presence of ACV and NGF	Removal of ACV, addition of PI3K inhibitor during continued NGF stimulation (761,762)
Infection of human embryo lung fibroblast or human or rat fetal neurons in the presence of interferon and an anti-HSV drug, e.g. acyclovir, followed by an increase in incubation temperature after removal of the inhibitor	Cold shock (465,477,500–502)
Primary sympathetic neuronal cultures, grown in the presence of NGF, infected with HSV	removal of NGF (502,763)
Infection of squamous epithelial cells cultured in a three-dimensional organotypic tissue culture (764)	

Table 15: HIV-1 accessory proteins in the capsid

Accessory protein	**Functions**
Vif	Forms E3 ubiquitin ligase complex to direct degradation of APOBEC3 proteins (96–101) Promotes ABOBEC3G virion exclusion in a degradation-independent manner, potentially by recruiting the restriction factor into high molecular weight RNA/protein masses that are unable to be encapsidated (432,512–514) Auxiliary factor for HIV-1 reverse transcriptase, increasing its rate of association to RNA or DNA templates (765,766) Depletes the intracellular pool of ABOBEC3G by directly binding ABOBEC3G mRNA and down-regulating its translation and stability (98,102,103) Directly inhibits ABOBEC3G deamination activity by altering its processive DNA scanning mechanism (104) Inhibits cell-cycle progression at the G2 phase of the cell cycle by interfering with Cdk1-CyclinB1 activation (524) Drives cells out of G1 and into the S phase of the cell cycle (523)
Vpr	Nuclear import of the preintegration complex (767) Induces TLR4/MyD88-mediated IL-6 production and reactivates HIV-1 virion production from latency (518) Impairs dendritic cell maturation, macrophage maturation and T-cell activation (768–770) Impairment of mitochondrial functions and induction of apoptosis (521,522) Induces G2 cell cycle arrest (525–527) Enhances expression from unintegrated HIV-1 DNA (551) Promotes NK cell-mediated killing (771,772) Transactivation of HIV-1 LTR expression by interacting with Sp1 (517) Increases hepatitis C virus RNA replication (520)
Nef	Downmodulates expression of several immune molecules, including CD4, MHC-I, MHC-II and C-C and C-X-C chemokine receptors (535,773,774) Modulates cellular secretory pathways to facilitate HIV-1 virion egress (775) Increases the efficiency of HIV-1 RT and facilitates proviral synthesis (776,777) Regulates viral protease activity (778) Alters macrophage function, for example by regulating the release of superoxide anions and inducing the secretion of chemotactic factors (779,780) Protects macrophages from HIV-1- and stress induced apoptosis (529–531) Activates AP-1, NFκB, STAT1 and STAT3 transcription factors (529,539–541) suppresses argonaute-2 function and causes large-scale dysregulation of cellular miRNAs (781,782) Prevents apoptosis of HIV-1-infected T cells through either interference with Fas/TNFαreceptor death-signalling pathways by inhibiting apoptosis signal-regulating kinase 1 (ASK-1), or by inactivating of the pro-apoptotic Bad protein (532–535) Promotes KSHV latency via regulation of cellular miRNA (783)